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International Union of Basic and Clinical Pharmacology. CX. Classification of Receptors for 5-hydroxytryptamine; Pharmacology and Function

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ABBREVIATIONS: AA, arachidonic acid; AC, adenylyl cyclase; AChE, Acetylcholinesterase; AD, Alzheimer disease; ADAR, RNA-specific adenosine deaminase; ADHD, attention deficiency hyperactivity disorder; AIM, abnormal involuntary movement; AIWG, antipsychotic-induced weight gain; AngII, Angiotensin II; AP-1, activator protein 1; AP-2, activator protein 2; AP-MS, affinity purification-mass spectrometry; APP, amyloid precursor protein; ARF1, ADP-ribosylation factor 1; ASD, autism spectrum disorder; AT1, angiotensin II receptor type 1; Aβ, amyloid-β; BAC, bacterial artificial chromosome; BDNF, brain-derived neutrophic factor; BNP, brain natriuretic peptide; BPAD, bipolar affective disorder; BSS, behavioral satiety sequence; CCI, chronic constriction injury; CD, cluster of differentiation; CDK5, cyclin-dependent kinase 5; cGMP, cyclic guanosine monophosphate; CGRP, calcitonin gene-related peptide; CHO, Chinese Hamster Ovary; CIPP, channel-interacting PDZ protein; cNOS, constitutive nitric-oxide synthase; CNS, central nervous system; CpG, methyl-cytosine-phosphate-guanine; CRMP, collapsing response mediator protein; 5-CT, 5-carboxamidotryptamine; CXCL, chemokine ligand; CYP, cyanopindolol; DA, dopamine; DAG, diacylglycerol; $DARPP_{32}$, dopamine and cAMP regulated phosphoprotein; DHE, dihydroergotamine; DOB, 2,5-Dimethoxy-4-bromoamphetamine; DOCA, deoxycorticosterone; DOI, 2,5-Dimethoxy-4-iodoamphetamine; DRG, dorsal root ganglion; DRN, dorsal raphe nucleus; EB, embryoid body; EC, enterochromaffin cells; ECD, extracellular domain; ECL2, extracellular loop 2; EEG, electroencephalography; ENS, enteric nervous system; EPS, extrapyramidal side effects; ER, endoplasmic reticulum; ERK, extracellular signal-regulated kinase; E3, ubiquitine E3 ligase; FDA, Food and Drug Administration; Fmr1, fragile X mental retardation 1; FST, forced swim test; FXS, fragile X syndrome; GASPs, GPCR-associated sorting proteins; GEF, guanine nucleotide exchange factor; GH, growth hormone; GI, gastrointestinal; GIP, glucose-dependent insulinotropic peptide; GIP, GPCR interacting protein; GIRK, G protein–coupled inwardly rectifying potassium channel; GLIC, gloeobacter ligand-gated ion channel; gp5-ht_{1e}, guinea pig 5-ht_{1e} receptor; GPCR, G protein–coupled receptor; GR, glucocorticoid receptor; GRK, G protein–coupled receptor kinase; GSK3, glycogen synthase kinase-3; HB-EGF, heparin-binding EGF-like growth factor; HCC, hepatocellular carcinoma; HDAC, histone deacetylase; 5-HIAA, 5-hydroxyindole acetic acid; HSC, hepatic stellate cells; 5-HT, 5-hydroxytryptamine; 5-HTBP, 5-HT binding protein; IBS, irritable bowel syndrome; IBS-C, IBS with constipation; IBS-D, IBS with diarrhea; ICC, interstitial cells of Cajal; ICD, intracellular domain; IDO, indoleamine 2,3-dioxygenase; IL, interleukin; IP_3, inositol-1,4,5-triphosphate; ISHH, in situ hybridization histochemistry; IUPHAR, International Union of Basic and Clinical Pharmacology; Jab, Jun activation domain–binding protein; JAK, Janus kinase; JCV, John Cunningham virus/polyomavirus; KO, knockout; L-DOPA, levodopa; LID, L-DOPA–induced dyskinesia; LNX, ligand of numb protein X; LPS, lipopolysaccharide; LSD, lysergic acid diethylamide; LTD, long-term depression; M1, proinflammatory macrophgase; M2, anti-inflammatory macrophage; MAGI2, membrane-associated guanylate kinase with inverted domain structure 2; Man, mannoses; MAP, microtubule-associated protein; MAP1B-LC1, light chain 1 subunit of MAP1B protein; MAPK, mitogen-activated protein kinase; mCPP, M-chlorophenylpiperazine; MDA, 3,4-methylene dioxyamphetamine; MDMA, 3,4-methylenedioxy methamphetamine; MeCP2, methyl-CpG-binding protein 2; miRNA, microRNA; MK, megakaryocyte; MMP, matrix metalloproteinase; mPFC, medial prefrontal cortex; MPP, MAGUK p55 subfamily member; MPP3, MAGUK p55 subfamily member 3; mTOR, mechanistic target of rapamycin; mTORC1, mTOR complex 1; MUPP1, multi-PDZ domain protein 1; NAc, nucleus accumbens; ND2, adaptor protein NADH dehydrogenase subunit 2; NF-κB, nuclear factor-κB; NHE-1, type 1 sodium-proton exchanger; NHERF, Na^+/H^+ exchanger regulatory factor; NK, neurokinin; NMDA, N-methyl-D-aspartic acid; NO, nitric oxide; NOS, nitric oxide synthase; nNOS, neuronal NOS; NOX, NADPH oxidase; NR1, NMDA receptor subunit 1; NREM, non–rapid eye movement sleep; OCD, obsessive-compulsive disorder; 8-OH DPAT, (±)-8-Hydroxy-2-dipropylaminotetralin; P2, purinergic 2 receptors; PAH, pulmonary arterial hypertension; PAM, positive allosteric modulator; PCP, phencyclidine; PCR, polymerase chain reaction; PD, Parkinson disease; PDE, phosphodiesterase; PDGFR, platelet-derived growth factor receptor; PDZ 10, MUPP1 PDZ domain 10; PDZ, PSD-95/Disc large/Zonula occludens; pEC_{50}, negative log of 50% effective concentration; PET, positron emission tomography; PFC, prefrontal cortex; PI3K, phosphatidylinositol-3 kinase; PKA, protein kinase A; PKC, protein kinase C; PLA_2, phospholipase A_2; PLAC-24, protein that localizes at cell-cell contacts; PLC, phospholipase C; PLD, phospholipase D; POMC, pro-opiomelanocortin; POV, postoperative vomiting; PPAR, peroxisome proliferator–activated receptors; PPI, PR, progressive ratio; PSD, postsynaptic marker; PSD-95, postsynaptic density-95; PTEN, phosphatase and tensin homolog deleted on chromosome 10; PX, phox homology; qPCR, quantitative polymerase chain reaction; REM, rapid eye movement sleep; Rheb, Ras homolog enriched in brain; RGS, regulator of G protein signaling; ROS, reactive oxygen species; RSK2, P90 ribosomal S6 kinase 2; RT-PCR, reverse transcriptase polymerase chain reaction; RTT, Rett syndrome; SAP97, synapse-associated protein 97; sAPP, soluble amyloid precursor protein; SAR, structure activity relationship; SCFAs, short-chain fatty acids; SERT, serotonin transporter; Sf9, spodoptera frugiperda 9 cells; siRNA, small-interfering RNA; SN, substantia nigra; snoRNA, small nucleolar RNA; SNP, single-nucleotide polymorphism; SNX, sorting nexin family member; SRE, serum response element; SRI, serotonin reuptake inhibitor; SSRI, selective serotonin reuptake inhibitor; STAT, signal transducer and activator of transcription; SW, slow wave; TASK-1, acid-sensitive potassium channel protein-1; TG, tissue transglutaminase; TGF-β1, transforming growth factor beta 1; Th, T-helper cells; THC, Δ^9-tetrahydrocannabinol; TLR, Toll-like receptors; TMD, transmembrane domain; TNAP, tissue nonspecific alkaline phosphatase; TNF, tumor necrosis factor; TPH, tryptophan hydroxylase; TSC, tuberous sclerosis; TST, tail suspension test; VTA, ventral tegmental area; WAVE-1, Wiskott-Aldrich syndrome protein family verprolin homologous protein 1; WT, wild type; Yif1B, Yip1 interacting factor homolog B; 2Cfl, full length 5-HT_{2C} receptor.

***Abstract*——5-HT receptors expressed throughout the human body are targets for established therapeutics and various drugs in development. Their diversity of structure and function reflects the important role 5-HT receptors play in physiologic and pathophysiological processes. The present review offers a framework for the official receptor nomenclature and a detailed understanding of each of the 14 5-HT receptor subtypes, their roles in the systems of the body, and, where appropriate, the (potential) utility of therapeutics targeting these receptors.**

***Significance Statement*——This review provides a comprehensive account of the classification and function of 5-hydroxytryptamine receptors, including how they are targeted for therapeutic benefit.**

I. Introduction

Classification of 5-HT receptors extends back to the middle of the last century when Gaddum and Picarelli (1957) suggested that the 5-HT–induced contraction of guinea pig ileum was mediated by two different receptors: a neurotropic "M" receptor located on parasympathetic ganglia (effect blocked by morphine and atropine; now known to be the 5-HT_3 receptor) and a musculotropic "D" receptor located on smooth muscles (effect blocked by dibenzyline, lysergide, 2-bromolysergide, and dihydroergotamine; now known to be the 5-HT_{2A} receptor). This original classification served well for around two decades, although, from time to time, it was reported that some 5-HT–induced effects (e.g., vasoconstriction in the canine carotid arterial bed) were not mediated by "M" or "D" but instead by "special" receptors (Saxena, 1974). Then, Bennett and Aghajanian (1974) reported the first successful radioligand binding study of 5-HT receptors using [^{3}H]lysergide, and subsequent studies using [^{3}H]5-HT, [^{3}H]spiperone, and [^{3}H]lysergide enabled Peroutka and Snyder (1979) to identify two 5-HT "receptors" named 5-HT_1 (nanomolar affinity for 5-HT) and 5-HT_2 (micromolar affinity for 5-HT). Subsequently, 5-HT_1 "receptors" were subdivided pharmacologically into 5-HT_{1A} and 5-HT_{1B} receptors (Pedigo et al., 1981), and 8-OH-DPAT was designated as a selective 5-HT_{1A} ligand (Gozlan et al., 1983; Middlemiss and Fozard, 1983). However, at these times, 5-HT receptors were being classified by various names (e.g., "D," "M," 5-HT_1, 5-HT_2, S_1, S_2), hence the clear need for uniform terminology. This effort culminated in the Bradley et al. (1986) publication, classifying 5-HT receptors into "5-HT_1-like" (equivalent to some "D" or 5-HT_1), 5-HT_2 (equivalent to most "D" or 5-HT_2), and 5-HT_3 (equivalent to "M") receptors. The authors emphasized that this classification was a "general framework," which would be regularly updated with new findings. Indeed, with the explosion in new findings around the time, it was clear a new classification was required that gave rise to the 5-HT receptor IUPHAR subcommittee–sanctioned classification of 5-HT receptors into 5-HT_1 ("5-HT_1-like," 5-HT_{1A}, 5-HT_{1B}, 5-HT_{1D}, 5-ht_{1e}, and 5-ht_{1f}), 5-HT_2 (5-HT_{2A}, 5-HT_{2B}, and 5-HT_{2C}), 5-HT_3, 5-HT_4, recombinant (5-$ht_{5a/5b}$, 5-ht_6, 5-ht_7), and "orphan" receptors (Hoyer et al., 1994). This new classification scheme was based on the conjunction of structural (molecular structure), transductional (intracellular transduction mechanisms), and operational (selective agonists and antagonists and ligand binding affinities) criteria. This first IUPHAR review on 5-HT receptors (Hoyer et al., 1994) was a landmark for the then rather complex 5-HT receptor field and the associated diversity of nomenclature used by operators in the field. In the 1994 review, we noted that the authors had a cumulated 100 years of active 5-HT research to share. A number of our colleagues have, in the meantime, retired from active research or have moved to other professional priorities. The present review provides a comprehensive overview of each of the recognized 5-HT receptors (Table 1) as well as reviewing the roles of 5-HT receptors in the major organs. There is a lot of new "blood" on board to reflect the growing diversity of the research, which is currently performed in many different academic and industrial centers; the combined years in 5-HT research of the present authors has increased considerably, partly because of the expansion of authors to ensure a comprehensive review of 5-HT receptors guided by the IUPHAR subcommittee on 5-HT receptors, which is chaired by Nicholas Barnes and Danny Hoyer.

In the present review, we address each receptor separately, as was performed previously, and then have sections that deal with specific aspects in more detail, such as the structures of 5-HT receptors, their functions in the major systems, and translational/clinical outcomes arising from 5-HT research. Readers are also directed to a website (http://www.guidetopharmacology.org/GRAC/FamilyDisplayForward?familyId=1) and the Concise Guide to Pharmacology (Alexander et al., 2019).

II. 5-HT_{1A} Receptor

A. Introduction

5-HT_{1A} receptors have attracted particular interest because of their negative feedback on 5-HT neurons,

TABLE 1
Nomenclature for 5-HT receptors

5-HT Receptor Groups	Nomenclature for 5-HT Receptors in the Group	Comments
5-HT_1 receptors		
	5-HT_{1A} receptor	
	5-HT_{1B} receptor	
	5-HT_{1D} receptor	
	5-ht_{1e} receptor	Lowercase appellation used by convention because a functional response in native cells or tissues has not been identified.
	5-HT_{1F} receptor	
5-HT_2 receptors		
	5-HT_{2A} receptor	
	5-HT_{2B} receptor	
	5-HT_{2C} receptor	
5-HT_3 receptors	Native receptors of unknown stoichiometry:	Five known subunits, 5-HT_{3A}, 5-HT_{3B}, 5-HT_{3C}, 5-HT_{3D}, and 5-HT_{3E}
	5-HT_3 receptor	5-HT_3 receptors, are pentameric complexes with the presence of
	Heterologous expression of known subunits such as	5-HT_{3A} subunits a prerequisite for function, i.e., only the
	Homomeric receptor:	homomeric 5-HT_{3A} receptor is functional. Heteromeric 5-HT_3
	5-HT_{3A} receptor	receptors are likely to require at least two 5-HT_{3A} subunits.
	Heteromeric receptor:	
	5-HT_{3AB} receptor	
	5-HT_{3AC} receptor	
5-HT_4 receptor	5-HT_4 receptor	
5-HT_5 receptors	5-HT_{5A} receptor	
	5-ht_{5b} receptor	Lowercase appellation is used by convention because a functional response in native cells or tissues has not been identified.
5-HT_6 receptor		
5-HT_7 receptor		

thus inhibiting 5-HT release and having broad influence on 5-HT tone. Additionally, 5-HT_{1A} receptors are widely distributed in terminal areas of the brain, where they are expressed as postsynaptic heteroceptors in a variety of different brain regions, influencing a range of neuropsychopharmacological sequalae (Albert and Fiori, 2014). After outlining the molecular structure, tissue expression, and the tools that can aid in the delineation of 5-HT_{1A} receptor function, the focus will be on the diverse therapeutic fields in which 5-HT_{1A} receptors have become a target. Accordingly, substantial efforts have focused on targeting 5-HT_{1A} receptors for pharmacotherapy of a variety of neurologic and psychiatric disorders, including major depressive disorder, anxiety, and schizophrenia. In addition, activation or blockade of 5-HT_{1A} receptors has been implicated in control of diverse other effects, including cognition, pain, fear, substance use disorder, and Parkinson disease (PD), and, more recently, in emerging clinical opportunities such as female sexual dysfunction and the treatment of respiratory deficits. The complexity of the effects of 5-HT_{1A} receptors presents both a challenge and a considerable opportunity for investigation of 5-HT function and for the potential identification of novel and improved therapeutic drugs.

B. 5-HT_{1A} Receptor Identification and Expression

The introduction of tritiated [^{3}H] receptor–binding techniques revealed the existence of 5-HT_1 (and 5-HT_2) receptor families in the prefrontal cortex (PFC) of the brain (Peroutka and Snyder, 1979), and extended studies indicated the existence of different 5-HT_1 receptor populations, designated, for the first time, 5-HT_{1A} and 5-HT_{1B} receptors (Pedigo et al., 1981; Middlemiss and Fozard, 1983), leading to a much greater understanding of the pharmacological and functional role of the 5-HT_{1A} receptor in health and disease.

The cloning of the 5-HT_{1A} receptor from various species confirmed the existence of 5-HT_{1A} receptors as distinct gene products that correlated with pharmacologically defined receptor responses (Table 2).

The 5-HT_{1A} receptor has been located in a wide variety of peripheral and central targets. In the periphery, immunohistochemical studies have demonstrated that the receptor is located in human and rat kidney, including medulla and cortical ascending limbs, the convoluted tubules, connecting tubule cells, and the principal cells of the initial collecting tubule (Raymond et al., 1993), and murine peritoneal macrophages (Freire-Garabal et al., 2003). However, other techniques have revealed a wider distribution: Western blotting found the receptor in human benign and malignant prostate tissue (Dizeyi et al., 2004), whereas reverse transcriptase polymerase chain reaction (RT-PCR) demonstrated the presence of 5-HT_{1A} receptors in rat taste buds (Kaya et al., 2004). However, the receptor is relatively poorly expressed in human coronary arteries, heart atrium, heart ventricles, and epicardium (Nilsson et al., 1999a,b). The brain and spinal cord have particularly dense populations of 5-HT_{1A} receptors, consistent with the role of this receptor in neuropsychiatric disease. The use of 5-HT_{1A} receptor agonists has been linked with the management of pain; accordingly, radioligand-binding and in situ hybridization studies have indicated the

TABLE 2
Overview of the amino acid structure, gene loci, and symbols and gene name of the human, mouse, rat, dog, and rhesus macaque monkey for 5-HT_{1A} receptors

High receptor homology, but homology in binding receptor domains is higher. Homologs described for other nonhuman primates, mosquito, *Gallus gallus domesticus*, *Danio rerio*, *Caenorhabditis elegans*, *Drosophila melanogaster*, *Bos taurus*, *Xenopus laevis*, *Cavia porcellus*, *Equus caballus*, etc. A total of 160 species have orthologs of the human receptor (https://www.ncbi.nlm.nih.gov/gene/?Term=ortholog_gene_3350[group]; http://www.ncbi.nlm.nih.gov/probe/?term=479890[unistsid]).

Species	Bp	AA	AA Homology with Human (%)	Chromosome Location	Gene Symbol
Human[a,b]	1269	422	100%	5q11.2-q13	HTR1A
Mouse[c]	1266	421	88%	13 D2.1	Htr1a
Rat[d,e]	1269	422	90%	2q16	Htr1a
Dog[f]	1272	423	92%	?	HTR1A
Monkey[g]	1266	421	98%	6.122.4cr	HTR1A

AA, number of amino acids; Bp, coding base pairs.
[a]Fargin et al., 1988.
[b]Stam et al., 1992.
[c]Charest et al., 1993.
[d]Albert et al., 1990.
[e]Fujiwara et al., 1990.
[f]Partly adapted from Andrade et al. (2019).
[g]Macaca mulatta.

presence in the human and rat dorsal and ventral horns (Pompeiano et al., 1992; Laporte et al., 1996) and rat superior cervical ganglia (Pierce et al., 1996). In the brain, a wide distribution of the receptor has been described in both terminal regions as postsynaptic sites and in the raphe nuclei, where it has a somatodendritic autoreceptor function (Jacobs and Azmitia, 1992; Fornal et al., 1994). Generally, there is much conservation in regional expression across species, although rat-human cortical and hippocampal differences in laminar organization were reported (Burnet et al., 1995; Barnes and Sharp, 1999). Within the brain, different techniques, including receptor binding, RT-PCR, in situ hybridization (Fig. 1), Western and Northern blotting, and immunohistochemistry, have localized the receptor to the septum, thalamus, hippocampus, entorhinal cortex, interpeduncular nucleus, olfactory bulb, amygdala, hypothalamic subnuclei, and subareas of the cortex and raphe nuclei (Gozlan et al., 1983; Hall et al., 1985; Pazos and Palacios, 1985; Weissmann-Nanopoulos et al., 1985; Dourish et al., 1986; Hoyer et al., 1986a; Vergé et al., 1986; Daval et al., 1987; Hamon et al., 1988; Albert et al., 1990; Hirose et al., 1990; Chalmers and Watson, 1991; Radja et al., 1991; Francis et al., 1992; Miquel et al., 1992; Pompeiano et al., 1992; Khawaja, 1995; Kung et al., 1995; Pike et al., 1995; Lemoine et al., 2010, 2012). More particularly, 5-HT_{1A} receptors are located on septal cholinergic neurons, cortical and hippocampal glutamatergic pyramidal neurons and granule cells (Francis et al., 1992; Pompeiano et al., 1992; Burnet et al., 1995), and calbindin- and parvalbumin-positive neurons (Aznar et al., 2003).

C. Pharmacology

In view of the involvement of 5-HT_{1A} receptors in a wide variety of physiologic responses, the pharmacological profile of these receptors has been investigated extensively using an impressive variety of ligands, with varying degrees of selectivity. These range from drugs preferentially targeting 5-HT_{1A} receptors to nonselective compounds that have broad pharmacological activities. Examples of the latter are atypical antipsychotic drugs such as clozapine, ziprasidone, or aripiprazole, which interact with many receptor subtypes. Notably, there are currently no selective 5-HT_{1A} receptor drugs approved for therapeutic use. This is somewhat surprising in view of the broad therapeutic interest of 5-HT_{1A} receptors but likely reflects the difficulty of identifying chemical scaffolds that selectively engage this target. For example, the anxiolytic agent, buspirone, and its chemical analogs such as ipsapirone and gepirone lack selectivity over some other receptors (for example, buspirone displays submicromolar affinity for dopamine D_2, D_3, and D_4 receptors; 5-HT_{2A}, 5-HT_{2B}, 5-HT_{2C}, 5-HT_6, and 5-HT_7 receptors; and α1 adrenoceptors). Similarly, several antagonist ligands have been proposed, but few have proved to be selective "silent antagonists." Nevertheless, some recent "full agonists" (notably befiradol) have been identified that exhibit good selectivity for 5-HT_{1A} receptors and, as such, may constitute first-in-class therapeutic agents.

Tables 3 and 4 summarize the receptor-binding properties of many 5-HT_{1A} receptor ligands that have been described over the last decades. It is also worth noting that even though certain compounds do display measurable receptor-binding affinity, this may be too low to induce functional responses at the 5-HT_{1A}

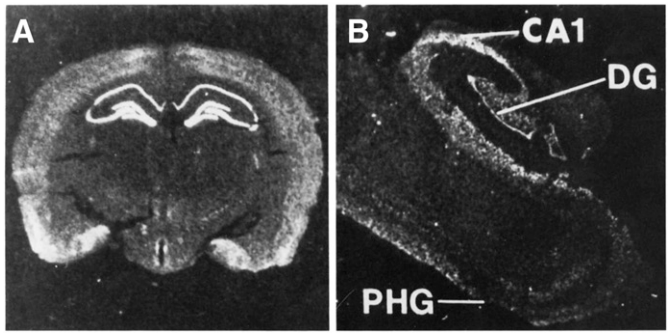

Fig. 1. In situ hybridization detection of 5-HT_{1A} receptor mRNA expression in rat (A) and human brain (B) at the level of the hippocampus. CA1, dentate gyrus (DG) of the hippocampus, and parahippocampal gyrus (PHG) are shown. Adapted from Burnet et al. (1995) (with permission).

receptor. Such an example is olanzapine, fails to elicit electrophysiological actions at the level of somatodendritic autoreceptors in contrast to ziprasidone and clozapine (Sprouse et al., 1999). Many of the ligands have been decisive in the operational definition of biochemical and pharmacological function at a basic science level and in key disease models. In addition to the receptor agonists and antagonists, there is some evidence for the existence of allosteric modulators, such as zinc, Galphimine-B, and RS-30199 (Spedding et al., 1998; Barrondo and Sallés, 2009; Jimenez-Ferrer et al., 2011).

The use of [^{35}S]GTPγS binding, a nonhydrolysable analog of GTP that binds to agonist-activated G proteins, has proved useful for investigating 5-HT$_{1A}$ receptor signaling and pharmacology (Newman-Tancredi et al., 1996b, 1997b, 1998; Barr and Manning, 1997; Pauwels et al., 1997; Sim et al., 1997; Stanton and Beer, 1997; Dupuis et al., 1999a,b; Cosi and Koek, 2000; Gonzalez-Maeso et al., 2000; McLoughlin and Strange, 2000; Shen et al., 2002; Odagaki and Toyoshima, 2005a,b, 2007). Notably, the use of [^{35}S]GTPγS binding enabled the investigation of both positive and negative efficacy ligands at 5-HT$_{1A}$ receptors. Thus, whereas a range of ligands efficaciously stimulated G proteins, other drugs, such as spiperone and methiothepin, markedly inhibited the [^{35}S]GTPγS basal binding in both membranes prepared from 5-HT$_{1A}$ receptor–transfected Chinese Hamster Ovary (CHO) cells and native tissue, confirming the capacity of 5-HT$_{1A}$ receptors to elicit constitutive activation of G proteins in vitro (Newman-Tancredi et al., 1997a; Stanton and Beer, 1997; McLoughlin and Strange, 2000; Corradetti et al., 2005; Martel et al., 2007). In contrast to spiperone, WAY1000635 exhibited neither positive nor negative efficacy yet blocked the actions of both agonists and inverse agonists, consistent with "neutral antagonist" properties (Fletcher et al., 1996; Martel et al., 2007) also evident in vivo using electrophysiological procedures (e.g., Fornal et al., 1996). This was important because other compounds claimed as antagonists at 5-HT$_{1A}$ receptors, such as NAN190, BMY7378, SDZ216,525, and even WAY100135, were found to display partial agonist properties when tested in systems that exhibit high degrees of receptor reserve (Greuel and Glaser, 1992; Routledge, 1996); changes in receptor expression level can markedly affect functional responses, and this is important when considering the nature of ligand engagement and the notion that different brain areas exert distinct physiologic influence (Newman-Tancredi et al., 1997c). A threefold increase in receptor:G protein ratio almost doubled relative efficacy of the partial agonist eltoprazine (53%–93%), without a change in potency, whereas 5-HT exhibited a twofold increase in potency (decrease in EC$_{50}$ value) (Newman-Tancredi et al., 1997c). In addition to these changes, the increase in 5-HT$_{1A}$ receptor:G protein ratio roughly doubled the negative efficacy of spiperone. These data therefore lead to the supposition that the targeting of agonist efficacy in vivo at different receptor populations is possible, which may offer therapeutic benefits.

D. Biased Agonism: Differential Activation of 5-HT$_{1A}$ Receptor Subpopulations

The term "biased agonism" ("functional selectivity" or "agonist-directed signaling") (Berg and Clarke, 2006; Evans et al., 2010; Kenakin, 2010; Tzingounis et al., 2010) was coined to denote a pattern of agonist signaling that was distinct from the concept of "intrinsic activity." Whereas the latter posits that receptor activation is an outcome of the "intrinsic" properties of the agonist, the concept of "biased agonism" is based on the capacity of agonists to preferentially mediate receptor signaling via specific pathways while not affecting, or even blocking, other secondary messenger pathways coupled to the same receptor. If the different signaling cascades mediate distinct functionality (e.g., therapeutic vs. side effects), then biased agonism will offer a strategy to potentially target different mechanisms with the opportunity to potentially develop more effective, better-tolerated drugs.

An early study of 5-HT$_{1A}$ receptors suggested that different agonists displayed differential Gαi2 and Gαi3 activation, determined using a photoreactive GTP analog (4-azidoanilido-[α-^{32}P]GTP) (Gettys et al., 1994). Rauwolscine displayed similar EC$_{50}$ values for activation of the two G protein subtypes; ipsapirone showed a nearly fourfold lower EC$_{50}$ for Gαi3 activation. 5-HT and 8-OH-DPAT had intermediate EC$_{50}$ values (Gettys et al., 1994). In another study, the presence of anti-Gαi3 antibodies almost completely suppressed G protein activation by pindolol, a 5-HT$_{1A}$ receptor partial agonist that preferentially elicits activation of Gαi3, a property that may underlie its preferential occupancy of midbrain 5-HT$_{1A}$ autoreceptors (Hirani et al., 2000; Martinez et al., 2001; Newman-Tancredi et al., 2002). Drug differences were also seen in transduction experiments on native rat raphe; buspirone elicited Gαi2-, Gαi3-, and Gαo-mediated responses as well as inhibition of adenylyl cyclase (AC), whereas 8-OH-DPAT only elicited coupling to Gαi3 and did not elicit the other responses (Valdizán et al., 2010).

Together, these data support that different 5-HT$_{1A}$ receptor agonists possess different G protein activation "fingerprints," backing the biased agonist concept and hence suggesting that 5-HT$_{1A}$ receptor subpopulation targeting is possible (Fig. 2). Compounds such as the biased 5-HT$_{1A}$ receptor agonists, F15599 and F13714, reversed immobility in the rat forced swim test via actions at presumed postsynaptic receptors. Similarly, anxiolytic-like actions were seen in the rat ultrasonic vocalization test (De Vry et al., 1993; Assié et al., 2010). However, in animal tests related to side effects, F15599 exhibited a better profile compared with F13714 (Gaggi et al., 1997; Prinssen et al., 2000; Assié et al., 2010), further supporting the potential for improved therapeutics utilizing biased agonists to target the appropriate

TABLE 3
Receptor-binding characteristics of 5-HT_{1A} receptor agonists

Data are extracted and adapted from Colpaert et al. (2002), Glennon et al. (2006), McCreary et al. (2007), Andrade et al. (2019), and McCreary and Newman-Tancredi (2019).

Agonist	Agonist Action	Affinity	Units	Clinical Utility
1-naphthylpiperazine	Full	8	pK_i	
5-CT	Full	9.4–10.3	pK_i	
5-hydroxytryptamine	Full	9.1–9.7	pK_i	
7-methoxy-1-naphthylpiperazine	Full	8.6	pK_i	
8-OH-DPAT	Full	8.4–9.4	pK_i	
(R)-UH 301	Partial	8.6	pK_i	
Adoprazine (SLV313)	Full/Partial	8.64–9.1	pK_i	Schizophrenia
Apomorphine	Partial	6.9	pK_i	PD, erectile dysfunction
Aripiprazole	Full	8.2	pK_i	Schizophrenia
Asenapine	Full	8.0–8.3	pK_i	Schizophrenia
BMY-7378	Partial	6.8–8.0	pIC_{50}	
BMY-1480	Full	7.2	pK_i	
Befiradol	Full	9.1	pKi	PD dyskinesia
Bifeprunox	Partial	7.19–8.95	pK_i	Schizophrenia
Brexpiprazole	Partial	9.92	pK_i	Schizophrenia
BRL-15572	Partial	7.7	pK_i	
Bromocriptine	Partial	7.9	pK_i	PD
Buspirone	Partial	7.7–8.0	pK_i	Anxiety
Cabergoline	Full	7.7	pK_i	PD
Capeserod	Partial	6	pK_i	
Cariprazine	Partial	8.59	pK_i	Schizophrenia
Clozapine	Full	6.8–6.9	pK_i	Schizophrenia
CP 93129	Full	6.1	pK_i	
Donitriptan	Full	7.6	pK_i	Migraine
Eletriptan	Full	7.4	pK_i	
Eltoprazine	Partial	8.03	pK_i	
EMDT	Full	6.8	pK_i	
F13714	Full	10.1	pKi	
F15063	Partial	8.24	pK_i	
F15599	Full	8.6	pKi	Rett syndrome
FG-5893	Full	8.7	pK_i	
Flesinoxan	Full	9.3	pK_i	Major depression
Flibanserin	Agonist	9	pK_i	Female hypoactive sexual desire
Fluparoxan	Partial	6.8	pK_i	
Frovatriptan	Agonist	7.2	pK_i	
GR127935	Partial	7.1–7.2	pK_i	
Ipsapirone	Partial	8.6–8.8	pK_i	
L-694,247	Full	9.3	pK_i	
L-772,405	Full	7.2	pIC_{50}	
Lisuride	Full	9.7–9.8	pK_i	Migraine
LP-12	Agonist	7.2	pK_i	
LP-44	Agonist	7.3	pK_i	
LP-211	Agonist	6.7	pK_i	
LSD	Full	9	pK_i	
Lurasidone	Full	8.17	pK_i	Schizophrenia
LY293284	Full	10.1	pK_i	
LY334370	Full	7.8	pK_i	
LY344864	Full	6.3	pK_i	
LY 165,163	Full	8.9	pK_i	
Nafadotride	Full	7.3	pK_i	
Naratriptan	Full	7.1–7.6	pK_i	Migraine
Nemonapride	Partial	8.35	pK_i	Schizophrenia
Ocaperidone	Full	8	pK_i	
Olanzapine	Full	5.6–5.8	pK_i	Schizophrenia
Pardoprunox (SLV308)	Full	8.5	pK_i	PD
Pergolide	Partial	8.7	pK_i	PD
Piribedil	Partial	6.4	pK_i	PD
Quetiapine	Full	6.5–6.6	pK_i	Schizophrenia
Quinpirole	Full	5.8	pK_i	
Repinotan	Full	9.4	pK_i	
Rizatriptan	Full	6.4	pK_i	Migraine
Roxindole	Partial	9.4–9.9	pK_i	
RU 24969	Full	9	pK_i	
S 16924	Partial	8.4	pK_i	
S-14506	Full	9.6–9.7	pK_i	
S-14671	Full	10.2–10.5	pK_i	
S-15535	Partial	9.2	pK_i	
Sarizotan	Partial	8.65	pKi	PD dyskinesia
SB 216641	Partial	6.3	pK_i	
Spiroxatrine	Full	8.8	pK_i	
SSR181507	Partial	8.53	pKi	
Sumatriptan	Full	6	pK_i	Migraine
Tandospirone	Partial	8.2	pK_i	Anxiety

(*continued*)

TABLE 3—*Continued*

Agonist	Agonist Action	Affinity	Units	Clinical Utility
Terguride	Partial	8.5	pK_i	PD
U92016A	Full	9.7	pK_i	
Vilazodone	Partial	9.7	pK_i	Depression
Vilazodone	Partial	9.5	pIC_{50}	Depression
Vortioxetine	Partial	7.8	pK_i	Depression
WAY-100135	Partial	8	pKi	
Xanomeline	Full	7.2	pK_i	
Zalospirone	Full	8.1	pK_i	
Ziprasidone	Partial	7.9–8.9	pK_i	Schizophrenia
Zolmitriptan	Full	6.6	pK_i	Migraine

EMDT, 2-Ethyl-5-methoxy-N,N-dimethyltryptamine.

5-HT_{1A} receptor subpopulation (Table 5), which includes potential to improve the cognitive state of patients with schizophrenia (Depoortère et al., 2010; Horiguchi and Meltzer, 2012).

E. 5-HT$_{1A}$ Receptor Intracellular Signal Transduction

The transfection (Fargin et al., 1988) and heterologous expression of 5-HT_{1A} receptors in various different cellular environments (including COS7, HeLa, CHO, NIH3T3, Sf9, and *Escherichia coli* cells) enabled the study of their G protein coupling to secondary messenger systems (Raymond et al., 1999). A well characterized intracellular functional response is the inhibition of AC activity and has been extensively used to differentiate ligands for this receptor, their agonist and partial agonist actions, or their degree of antagonism (De Vivo and Maayani, 1986; Markstein et al., 1986; Bockaert et al., 1987; Shenker et al., 1987; Dumuis et al., 1988b; Fargin et al., 1989; Varrault and Bockaert, 1992; Raymond et al., 2006). 5-HT_{1A} receptors can also activate G protein inward rectifying potassium channels (GIRK), high-conductance anion channels to inhibit calcium conductance modulating intracellular calcium mobilization, and stimulate nitric oxide synthase (NOS) and an NADP oxidase-like enzyme (Adayev et al., 2003; Hsiung et al., 2005; Polter and Li, 2010). The receptor can affect metabolism and arachidonic acid (AA) production (Raymond et al., 1999); activate protein kinase C production, Src kinase, and mitogen-activated protein kinases (MAPKs); and activate or inhibit phosphoinositol hydrolysis and stimulate reactive oxygen species (ROS) production (superoxide and peroxide) (Raymond et al., 1999). Together, the elucidation of this diverse pattern has led to important developments in establishing test systems to probe receptor and drug function.

F. Function

1. Differential Function of 5-HT$_{1A}$ Receptors at Cellular, Tissue, and In Vivo Levels. The functional properties of 5-HT_{1A} receptors have been extensively investigated. The overall conclusion from these studies is that subpopulations of 5-HT_{1A} receptors expressed in different brain regions exhibit specific patterns of receptor signaling, with differing impact on central function. These diverse properties indicate that separate subpopulations of 5-HT_{1A} receptors mediate particular responses and may constitute therapeutic targets in their own right (see also Fig. 2). For example, agonist activation of somatodendritic 5-HT_{1A} autoreceptors expressed on serotonergic neurons in the raphe elicits inhibition of 5-HT release in terminal regions such

TABLE 4
Receptor-binding characteristics of 5-HT_{1A} receptor antagonists
Table adapted from Andrade et al. (2019).

Antagonist	Affinity	Units
(+)-butaclamol	6.4	pK_i
(-)-propranolol	7.5	pK_i
(-)-tertatolol	8.2	pK_i
(R)-flurocarazolol	6.5	pK_i
(S)-flurocarazolol	7.5	pK_i
(S)-UH 301	7.9	pK_i
[^{3}H]p-MPPF	8.4	pK_d
[^{3}H]robalzotan	9.8	pK_d
[^{3}H]WAY100635	9.5	pK_d
[^{11}C]WAY100635	—	—
Chlorpromazine	6.2	pK_i
Cyamemazine	6.3	pK_i
Fluspirilene	7.2	pK_i
GR 125,743	7.3	pK_i
GR 218,231	6.8	pK_i
Haloperidol	5.7–5.8	pK_i
Iloperidone	6.8–7	pK_i
Ketanserin	5	pK_i
Mesoridazine	7	pK_i
Methiothepin	7.8–8.1	pK_i
MPDT	5.8	pK_i
NAN 190	9.4	pK_i
p-[^{18}F]MPPF	—	—
p-MPPI	8.4	pK_i
Pimozide	6.8	pK_i
Pindolol	8.1	pK_i
Pipamperone	5.6	pK_i
Pizotifen	7.4	pK_i
Raclopride	5.2	pK_i
Rec 15/3079	9.7	pK_i
Risperidone	6.2–6.5	pK_i
9-OH-risperidone	6.2	pK_i
ritanserin	5.2–5.5	pIC_{50}
robalzotan	9.2	pK_i
SB 272183	8	pK_i
SB 649915	8.6	pK_i
SB 714786	6.5	pK_i
SDZ-216525	7.8–8.2	pIC_{50}
Sertindole	6.4–6.6	pK_i
Spiperone	6.7–8.8	pK_i
Thioridazine	7.1	pK_i
Tiospirone	8.3	pK_i
WAY-100635	7.9–9.2	pK_i
Yohimbine	7.3	pK_i
Zotepine	6.5	pK_i

MPPF, 2'-methoxyphenyl–p-fluoro-benzamidoethyipiperazine.

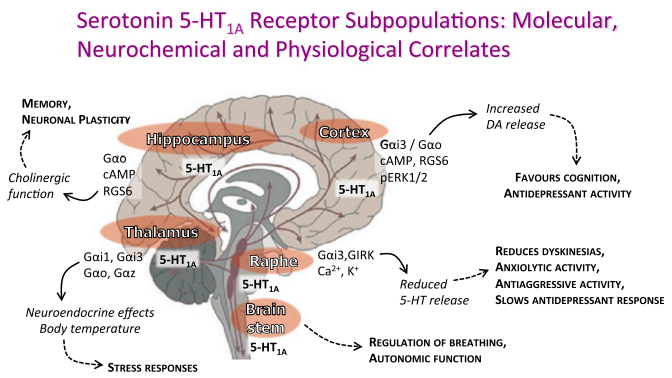

Fig. 2. Biased agonism at the 5-HT_{1A} receptor offers the potential to target subpopulations of 5-HT_{1A} receptors.

as the hippocampus and cortex. In contrast, activation of postsynaptic cortical 5-HT_{1A} heteroreceptors expressed on glutamatergic pyramidal cells and/or GABAergic interneurons elicits different neurochemical responses, including stimulation of dopamine release in the frontal cortex (Santana et al., 2004; Bortolozzi et al., 2010).

Activation of 5-HT_{1A} autoreceptors induces anxiolytic activity in rodent behavioral tests (De Vry et al., 2004; Akimova et al., 2009), whereas antidepressant-like responses are seen upon activation of 5-HT_{1A} heteroceptors (De Vry et al., 2004). These data obtained in rat behavioral experiments are consistent with observations in transgenic mice overexpressing raphe 5-HT_{1A} autoreceptors; accentuated depressive-like behavior was observed and diminished response to antidepressant treatment (Richardson-Jones et al., 2010). These data support the interpretation that desensitization of presynaptic 5-HT_{1A} receptors is necessary before antidepressant efficacy may be achieved (Artigas et al., 2006; Millan, 2006), consistent with the relatively long latency (typically 3 to 4 weeks) to clinical responsivity in patients with depression treated with 5-HT reuptake inhibitors.

Diverse responses to 5-HT_{1A} receptor agonists are also observed in tests of cognition/memory function relevant to numerous neuropsychiatric diseases, including major depressive disorder, schizophrenia, Parkinson disease, and Alzheimer disease. Interestingly, the prototypical 5-HT_{1A} receptor agonist, 8-OH-DPAT, facilitated rat passive avoidance at low doses, whereas higher doses impaired performance (Lüttgen et al., 2005; Madjid et al., 2006). This suggests that opposite responses are mediated by 5-HT_{1A} receptor subpopulations (i.e., improved performance is elicited by 5-HT_{1A} autoreceptors, whereas impairment is due to activation of hippocampal 5-HT_{1A} heteroreceptors) (Ogren et al., 2008). This interpretation is supported by local administration experiments in which the 5-HT_{1A} receptor weak partial agonist/antagonist S15535 was microinjected into the hippocampus. The compound reversed the memory deficit elicited by systemic injection of 8-OH-DPAT in a spatial discrimination task (Millan et al., 2004), indicating that activation of postsynaptic receptors in this brain region was detrimental to mnesic performance.

Given that only a single 5-HT_{1A} receptor gene has been identified in human and rat, and that it is intronless and hence without splice variants (Fargin

TABLE 5

Comparison of properties of 5-HT_{1A} receptor "biased agonists" F15599, F13714, and befiradol, and the reference agonists 8-OH-DPAT and 5-HT

Based on the publications indicated below. Target brain regions are those identified in microPET imaging and neurochemical experiments. In vivo readouts are nonexhaustive and focus on the primary activities of the biased agonists.

Agonist	In Vitro Affinity/Selectivity[a]	Cellular Transduction Pathways[b]	Target Brain Regions[c]	Readout In Vivo[d]	Relevant Therapeutic Indications
F15599	Nanomolar/highly selective	Preferential pERK activation	Cortex, brain stem	Reverses PCP-induced cognitive deficits, active in antidepressant and anxiolytic tests. Normalizes breathing in $MeCP2^{+/-}$ mice	Cognitive deficits, mood disorders, respiratory difficulties (Rett syndrome)
F13714	Subnanomolar/highly selective	Multiple (pERK, receptor internalization, G protein, cAMP, Ca^{2+} release)	Mid-brain, thalamus, hippocampus,	Potently eliminates L-DOPA–induced AIMs, active in antidepressant and anxiolytic tests	Not clinically tested (research tool)
Befiradol	Nanomolar/highly selective	Multiple (pERK, receptor internalization, G protein, cAMP, Ca^{2+} release)	Mid-brain, cortex, thalamus, hippocampus	Potently eliminates L-DOPA–induced AIMs, active in antidepressant and anxiolytic tests	Dyskinesias in Parkinson disease, mood deficits, chronic pain
8-OH-DPAT	Nanomolar/binds 5-HT_7	Preferential pERK activation	Hippocampus, mid-brain, cortex, thalamus, brain stem	Disparate effects on cognition tests, active in antidepressant and anxiolytic tests, reduces L-DOPA–induced AIMs	Not clinically tested (research tool)
5-HT	Nanomolar/ nonselective	Multiple (pERK, receptor internalization, G protein, cAMP, Ca^{2+} release)	All 5-HT projection areas		N/A

N/A, not applicable.
[a]Colpaert et al., 2002; Newman-Tancredi et al., 2009a.
[b]Colpaert et al., 2002; Pauwels and Colpaert, 2003; Buritova et al., 2009; Newman-Tancredi et al., 2009b.
[c]Lemoine et al., 2010, 2012; Lladó-Pelfort et al., 2010, 2012; Vidal et al., 2014.
[d]Assié et al., 2010; Depoortere et al., 2010; Levitt et al., 2013; Iderberg et al., 2015; van Goethem et al., 2015.

et al., 1988; Albert et al., 1990; Kobilka et al., 1987), the variety of responses described above are likely attributable to regional "receptor interactome" differences, including coupling to distinct G protein subtypes (see below) (Mannoury la Cour et al., 2006), regulators of G protein signaling (RGS) (Talbot et al., 2010), or transcriptional regulation.

At a molecular level, 5-HT_{1A} receptor inactivation studies using N-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline demonstrated the existence of receptor reserve in the raphe for inhibition of 5-HT synthesis (Meller et al., 1990). In contrast, receptor reserve was not evident in the hippocampus for the inhibition of adenylyl cyclase or for control of hypothermia (Meller et al., 1992; Yocca et al., 1992).

The agonist radioligand, [^{3}H]8-OH-DPAT, which preferentially recognizes 5-HT_{1A} receptors when coupled to G proteins, displayed fivefold higher affinity for hippocampal compared with raphe binding sites (Johnson et al., 1997b), supporting differing receptor–G protein coupling state between the two brain regions. Furthermore, whereas 5-HT_{1A} receptors are coupled to inhibition of adenylyl cyclase in hippocampus, such coupling was not detected in raphe homogenates (Clarke et al., 1996). Further support that 5-HT_{1A} receptors couple to different G protein subtypes depending on brain region arises from immunoprecipitation studies in which raphe 5-HT_{1A} receptors couple preferentially to Gαi3 subtypes, whereas they couple preferentially to Gαo in hippocampus and to a combination of G proteins in cortex and hypothalamus (Mannoury la Cour et al., 2006).

Functional ([^{35}S]GTPγS) autoradiography experiments also support the contention that regional variations exist in native brain activation of G proteins by 5-HT_{1A} receptors. Indeed, whereas 5-HT_{1A} receptor density is similar in the raphe and hippocampus, agonist-induced [^{35}S]GTPγS labeling was markedly lower in the former [Hensler, 2003; see also Newman-Tancredi et al. (2003) for relevant evidence].

An additional level of complexity of 5-HT_{1A} receptor signaling has been reported (i.e., the existence of receptor homo-, hetero-, and potential trimers with a variety of targets). First, 5-HT_{1A} homodimers may be formed constitutively (Łukasiewicz et al., 2007; Renner et al., 2012) and are affected by the presence of selective ligands such as 8-OH-DPAT, which enhanced dimerization, whereas methysergide reduced dimer formation potentially via a mechanism modulated by Gαs subunits. The 5-HT_7 receptor, like the 5-HT_{1A} receptor, has been reported to play a role in depression and form homodimers; but it has also heterodimerized with the 5-HT_{1A} receptor and may have functional consequences insofar as 5-HT_{1A}–5-HT_7 heterodimerization reduces GIRK currents in a heterologous cell system, potentially affecting 5-HT_{1A} receptor internalization in the hippocampus (Renner et al., 2012; Naumenko et al., 2014). Heterodimerization with a novel negative response element of 5-HT_{1A} receptors has been suggested with glucocorticoid and mineralocorticoid receptors, which may also be key players in depression (Ou et al., 2001). Galanin receptors form heteromers with a variety of targets, including galanin receptor–5-HT_{1A} heteromers and trimers (Fuxe et al., 2012). With potential relevance to the influence of 5-HT_{1A} receptors in ascending and central pain perception, heterodimerization has also been demonstrated with 5-HT_{1A} receptors and μ-opioid receptors in vitro, and further data suggested that both receptors could exert effects on extracellular signal–regulated kinase (ERK)1/2 phosphorylation (Cussac et al., 2012).

a. 5-HT_{1A} receptors in depression and anxiety. The key role of 5-HT_{1A} receptors in major depression and anxiety has been recognized for nearly four decades [see Barnes and Sharp (1999); Albert et al. (2014)]. Accordingly, animal behavioral models of fear, anxiety, depression, and cognition have been used to identify potential antidepressant and clinically active anxiolytics, such as the partial agonists buspirone, gepirone, ipsapirone, and tandospirone (Peroutka, 1985; Taylor et al., 1985; Gilbert and Dourish, 1987). Buspirone and tandospirone were both clinically developed and received marketing approval for treating anxiety. However, their azapirone chemical structures are associated with only limited selectivity (e.g., vs. α_1 adrenoceptors and D_2 dopamine receptors), and they also exhibit relatively poor metabolic stability and generate pharmacologically active metabolites, such as 1-(2-pyrimidinyl)-piperazine, which is an α_2 adrenoceptor antagonist (Garattini et al., 1982; Cao and Rodgers, 1997; Zuideveld et al., 2002; Sugimoto et al., 2005; Wong et al., 2007). Consequently, the therapeutic usefulness of selective 5-HT_{1A} receptor agonists still remains to be determined.

Indeed, data suggest that the phenotypic expression of normal behavior, anxiety, or depression may be influenced by the differential 5-HT_{1A} receptor–sensitive circuitry at the level of the PFC; the balance between 5-HT_{1A} receptor stimulation of glutamatergic pyramidal cells and GABAergic interneurons may impact the expression of anxiety (Goodfellow et al., 2009; Albert et al., 2014), although juvenile development processes play a key role in determining vulnerability to mood disorders (Leonardo and Hen, 2008; Donaldson et al., 2014; Garcia-Garcia et al., 2014). In depression, the neurobiology appears different. Therefore, activation of pyramidal neurons by stimulation of 5-HT_{1A} receptors expressed on GABAergic interneurons disinhibits the "antidepressive" pyramidal neurons (Albert et al., 2014). It is interesting to note that the rapid antidepressant activity of ketamine appears to be partly mediated via 5-HT_{1A} receptor activation. Indeed, ketamine inhibits 5-HT reuptake in vivo (Martin et al., 1982; Martin and Smith, 1982) and elicits its prolonged antidepressant-like effects in rodents via a 5-HT–dependent mechanism (Gigliucci et al., 2013). This is likely to involve indirect activation of 5-HT_{1A} receptors,

as exemplified by the fact that the effects of ketamine in the novelty-suppressed feeding test are blocked by a 5-HT_{1A} receptor antagonist (Fukumoto et al., 2014).

Additional evidence that 5-HT_{1A} receptors are involved in affective disorders comes from genetic studies. The expression of 5-HT_{1A} receptors is differentially regulated by a single-nucleotide polymorphism (SNP) in the promoter region of the 5-HT_{1A} receptor gene (C-1019G substitution) (Lesch and Gutknecht, 2004; Albert and Francois, 2010). This SNP impairs repression of the 5-HT_{1A} promoter by the nuclear DEAF-1-related/drosophila deformed epidermal autoregulatory factor-1 transcription factors in raphe cells, consistent with overexpression of presynaptic 5-HT_{1A} receptors (Lemonde et al., 2004; Parsey et al., 2006). Thus, C-1019G polymorphism is associated with higher levels of symptom remission failure and suicidal behavior in patients with depression (Lemonde et al., 2003), consistent with impaired antidepressant efficacy caused by excessive feedback inhibition by presynaptic 5-HT_{1A} receptors.

Taken together, the above considerations indicate that 5-HT_{1A} receptors remain promising targets for the pharmacotherapy of affective disorders, both as a somatodendritic and postsynaptic receptor target in the brain. Accordingly, various efforts have been made to incorporate 5-HT_{1A} receptor activity in antidepressant/anxiolytic drug candidates For example, SB-649915-B is a 5-HT reuptake inhibitor (SSRI) that also acts as a 5-HT_{1A} receptor antagonist (Hughes et al., 2007; Starr et al., 2007) based on the rationale that accelerated antidepressant response may be achieved by avoiding feedback inhibition of terminal 5-HT release by blocking the activation of 5-HT_{1A} autoreceptors (Gartside et al., 1999; Artigas et al., 2006; Portella et al., 2011). However, though antidepressant efficacy may be enhanced by 5-HT_{1A} autoreceptor antagonism, the blockade of postsynaptic 5-HT_{1A} receptors likely opposes antidepressant activity (De Vry et al., 2004; Berrocoso and Mico, 2009). Accordingly, a clinical trial in which a selective 5-HT_{1A} receptor antagonist was administered as adjunct to fluoxetine did not show any acceleration of antidepressant onset of efficacy (Scorza et al., 2012), likely because of its concurrent blockade of both pre- and postsynaptic 5-HT_{1A} receptors. In contrast, adjunct treatment with pindolol, which preferentially occupies 5-HT_{1A} autoreceptors (Martinez et al., 2001), appears to reliably elicit acceleration of antidepressant efficacy (Artigas et al., 1996, 2006; Portella et al., 2011). Compounds such vilazodone, vortioxetine, and VN2222 are SRIs possessing partial agonist actions at 5-HT_{1A} receptors (Romero et al., 2003; Dawson and Watson, 2009; Mork et al., 2009; Alvarez et al., 2012) that might assist in engaging diverse frontal circuitry, leading to better treatment of the disease.

b. 5-HT_{1A} receptor activation for improved antipsychotic action. A noteworthy development in the study of 5-HT_{1A} receptors has been the increasing therapeutic interest for this target in psychotic disorders. This has stemmed from extensive clinical and preclinical observations [see McCreary and Newman-Tancredi (2015) for review].

Schizophrenia, which shares some symptoms with other neuropsychiatric diseases, includes positive symptoms (auditory and visual hallucinations, delusions, conceptual disorganization, thought disorders, and some motor disturbances); negative symptoms (affective blunting, social withdrawal, anhedonia, avolition, and poverty of thought and speech); and cognitive impairments, such as working-memory abnormalities, deficits of cognitive processing, and attention and affective disorders (depression and anxiety) (Meltzer, 1999). 5-HT_{1A} receptors appear involved both in the pathophysiology and in functionality of potential novel treatments. Thus, the newer generation antipsychotics clozapine, ziprasidone, quetiapine, aripiprazole, lurasidone, and cariprazine possess (partial) agonist effects at 5-HT_{1A} receptors; however, interestingly, risperidone and olanzapine do not (McCreary and Newman-Tancredi, 2015; Newman-Tancredi et al., 1996a, 2005). In patients, changes in 5-HT_{1A} receptor binding or functional activity have been identified (Burnet et al., 1996; Kasper et al., 2002; Yasuno et al., 2003; Bantick et al., 2004; Frankle et al., 2006; Lerond et al., 2013; Billard et al., 2014) along with SNPs at loci ss212928868 and rs6294, which are associated with the clinical outcome in women with paranoid schizophrenia (Zhou et al., 2013). Polymorphisms were also associated with much of the depression and negative treatment outcomes (Reynolds et al., 2006; Newman-Tancredi and Albert, 2012). Preliminary studies assessing cytosine methylation at a site close to this rs6295 polymorphism suggested that this was associated with a lower incidence of negative symptoms (Reynolds et al., 2006; Tang et al., 2014b), reinforcing the importance of this site in the negative symptoms of schizophrenia. Taken together, these accumulated data support the assertion that there is involvement of 5-HT_{1A} receptors in the pathophysiology and treatment-related facets of the disease, particularly negative symptomatology.

A net hypofunctionality of the PFC, a brain area key in working memory, decision, and attentional processing, has been proposed in schizophrenia (Weinberger and Lipska, 1995; McCreary et al., 2007). It is therefore interesting that many atypical antipsychotic drugs may impact this deficit (McCreary and Newman-Tancredi, 2015). It may therefore be relevant that the 5-HT_{1A} receptor agonist agents possessing antipsychotic properties (SSR181507, adoprazine, and lurasidone) augment extracellular microdialysate dopamine and acetylcholine levels in the PFC to "normalize" hypofrontal tone (Claustre et al., 2003; McCreary et al., 2007; Huang et al., 2014b) and promote potential therapeutic outcomes. This is supported by preclinical evidence (Depoortère et al., 2007) and clinical evidence with the partial agonist, tandospirone, which improved cognitive symptoms in patients with schizophrenia treated with

neuroleptics (Sumiyoshi et al., 2001a,b, 2007; Meltzer and Sumiyoshi, 2008). Additionally, blonanserin, tandospirone, lurasidone, and buspirone reduced MK-801–induced novel object recognition deficits (Horiguchi and Meltzer, 2012; Horiguchi and Meltzer, 2013), and PCP-induced reversal learning was attenuated by 5-HT_{1A} receptor activation (McLean et al., 2009b). In the social interaction test, a model for negative symptoms, aripiprazole, SSR181507, and F-15063 induced a 5-HT_{1A} receptor–dependent performance improvement (Boulay et al., 2004; Bruins Slot et al., 2005; Depoortère et al., 2007; Snigdha and Neill, 2008). In addition, administration of 5-HT_{1A} receptor (partial) agonists reversed PCP-induced decreases of tickling-induced 50-kHz ultrasound vocalization in juvenile rats, a model for negative symptoms, and improved attentional processing in a five-choice serial reaction time task (Winstanley et al., 2003; Boulay et al., 2013). In conclusion, data from preclinical and clinical findings support that 5-HT_{1A} receptor activation will benefit the treatment of cognitive, attentional, and negative symptom domains.

An additional complication of antipsychotic treatment is so-called extrapyramidal side effects induced by the typical antipsychotics, such as haloperidol, which can reduce striatal output and lead to a parkinsonian phenotype. Such symptoms in preclinical models can be reduced by 5-HT_{1A} receptor agonists (McCreary et al., 2007).Compounds such as adoprazine, bifeprunox, and F-15063 elicit less catalepsy than neuroleptics such as haloperidol. However, treatment, with WAY-100635 unmasked this blockade of catalepsy, indicating a key role of 5-HT_{1A} receptors (Kleven et al., 2005; Bardin et al., 2006). Consistently, mesolimbic selectivity, and therefore the ability to treat the positive symptoms, was supported with electrophysiological studies demonstrating that depolarization block of VTA, but not substantia nigra pars compacta, dopaminergic neurons was mediated by 5-HT_{1A} receptor agonists (Nakamura et al., 2006; McCreary et al., 2007) and that PFC 5-HT_{1A} receptors influenced VTA cell firing by indirectly affecting pyramidal cell afferents to the VTA, thereby increasing dopamine cell firing (Lladó-Pelfort et al., 2012; Santana et al., 2013). Such mechanisms may indirectly influence mesoaccumbal dopaminergic output and impact positive symptoms. Some clinical meta-analytical studies support this assertion and suggest a trend for improved cognitive symptoms following the addition of 5-HT_{1A} receptor partial agonists, together with a trend for improved positive symptoms (Kishi et al., 2013), but more extensive clinical studies are warranted. It is interesting to speculate that fully efficacious agents might offer added benefit. Moreover, benefit in other symptom domains might be expected, particularly mood. Accordingly, bifeprunox, SSR181507, and adoprazine (SLV313) all demonstrated anxiolytic-like and antidepressive-like properties (Depoortere et al., 2003), and 5-HT_{1A} receptors appear to mediate the antidepressant effects of ketamine and metabotropic glutamate (2/3) receptor antagonists (Fukumoto et al., 2014). Moreover, 5-HT_{1A} gene loci polymorphism linkage studies support this in schizophrenic patients with depression (Albert, 2012).

Taken together, these data support a role for the 5-HT_{1A} receptor in schizophrenia. This is particularly interesting in light of the clinical development and marketing approval of lurasidone and cariprazine, which possess dopamine D_2 and 5-HT_{1A} receptor agonist action (Ishibashi et al., 2010; Kiss et al., 2010). Indeed, pharmacodynamic studies support the described 5-HT_{1A} receptor–mediated mechanisms in the actions of lurasidone on augmented PFC dopamine and acetylcholine levels and cognitive actions (Horiguchi and Meltzer, 2012; Huang et al., 2012, 2014). Consistently, clinical benefit in a variety of symptom domains was evident (Veselinović et al., 2013; Citrome et al., 2014; Durgam et al., 2014; Loebel et al., 2014a,b).

G. 5-HT_{1A} Receptors and Some Emerging Treatment Areas

1. Parkinson Disease. Parkinson disease is characterized by a loss of nigrostriatal dopaminergic neurons, resulting in the cardinal motor symptoms (Schapira et al., 2006). Symptomatic treatment ultimately relies on the gold-standard medication and dopamine precursor levodopa (L-DOPA) (Jenner et al., 2011). However, over time, the effects of L-DOPA are prone to wearing off (i.e., there is a tolerance to the actions of L-DOPA), and patients develop dose-limiting dyskinesia (Jenner et al., 2011). The treatment of L-DOPA–induced dyskinesia (LID) has been hampered by a lack of approved medications. Recently, the 5-HT system has emerged as a key player in the induction of LID. 5-HT neurons possess the enzymes necessary to convert exogenous L-DOPA to dopamine (DA) and mediate its vesicular storage and "false neurotransmitter" release. However, 5-HT neurons lack appropriate control mechanisms to regulate synaptic DA levels (e.g., via presynaptic D_2 receptors or dopamine transporters), resulting in excessive DA release and pulsatile (over) stimulation of postsynaptic dopamine receptors that generate dyskinesia. Theoretically, it might be possible to mitigate dopamine release from serotonergic neurons by suppressing serotonergic tone by the application of 5-HT_{1A} (or 5-HT_{1B}) receptor agonists, which suppress neurotransmission by influencing the negative feedback somatodendritic (or terminal autoreceptors). Indeed 5-HT_{1A} receptor agonist treatment does reduce LID in both rat and nonhuman primate models (Bibbiani et al., 2001; Eskow et al., 2007, 2009; Munoz et al., 2009; Huot, 2015; Iderberg et al., 2015) and appears to translate in clinical studies using the partial agonists buspirone and the mixed 5-HT_{1A}/5-HT_{1B} agonist eltoprazine (Svenningsson et al., 2015). However, other clinical attempts to target the 5-HT_{1A} receptor have been disappointing,

with compounds such as sarizotan and tandospirone also impairing the antiparkinsonian activity (Bonifati et al., 1994; Kannari et al., 2002; Olanow et al., 2004; Goetz et al., 2007), whereas eltoprazine showed only modest effects (Svenningsson et al., 2015). Together, this suggests that although 5-HT_{1A} receptors can reduce dyskinesia, compounds tested to date may be less than optimal (Hamik et al., 1990; Newman-Tancredi et al., 1997c, 1998, 2003). Interestingly, only full agonists succeed in completely reversing haloperidol-induced catalepsy, whereas partial agonists failed to do so (Prinssen et al., 2002), suggesting that maximal efficacy may be required. The selective 5-HT_{1A} receptor "biased agonist" F13714, which preferentially targets raphe 5-HT_{1A} autoreceptors (Assié et al., 2006), completely abolished abnormal involuntary movements (AIMs) along with inhibiting 5-HT release (Iderberg et al., 2015). Comparable findings were evident with Befiradol (McCreary and Newman-Tancredi, 2015).

In addition, "full agonist" activity at 5-HT_{1A} receptors may also provide beneficial influence on nonmotor symptoms of PD, such as the mood deficits likely elicited by deficient 5-HT neurotransmission (Eskow Jaunarajs et al., 2010; Politis, 2010). Indeed, whereas treatment of depressive symptoms in PD using 5-HT reuptake inhibitors is poorly effective, direct activation of postsynaptic (cortical) 5-HT_{1A} receptors is associated with potent antidepressant actions (Celada et al., 2004). In restless legs syndrome, another movement disorder typically managed with low doses of dopamine receptor agonists or L-DOPA, 5-HT_{1A} receptor agonists may also display clinical benefit (Shioda et al., 2006).

2. Pain. There is good evidence for the involvement of the 5-HT system in chronic pain (Millan, 2002), which is not surprising given their expression by descending pathways of the dorsal horn and other relevant structures. The receptors of the dorsal horn appear pivotally involved in the pronociceptive effects (Fasmer et al., 1986; Millan, 1994, 2002; Millan et al., 1996; You et al., 2005; Colpaert, 2006; Avila-Rojas et al., 2015; Sagalajev et al., 2015) and may also influence antinociception (Millan et al., 1996). Recent evidence suggests that the newer generation antipsychotic agent (e.g., aripiprazole), which possesses 5-HT_{1A} receptor partial agonist actions, displays antinociceptive effects (Fei et al., 2012; Almeida-Santos et al., 2015). Moreover, the ability of 5-HT_{1A} receptors to form heterodimers with μ-opioid receptors (Cussac et al., 2012) suggests 5-HT_{1A} receptor targeting as an adjunct to opioid strategies may be useful.

3. Attention Deficiency Hyperactivity Disorder. In animal models of impulse control, 5-HT_{1A} receptor stimulation reduced the impulsivity, suggesting potential benefit in diseases such as attention deficiency hyperactivity disorder (ADHD; Winstanley et al., 2003). Furthermore, in an isolation rearing model, which models some components of ADHD, 5-HT_{1A} receptor binding sites were altered in a region-specific manner (Preece et al., 2004). Pharmacological study using the agonists SSR181507 (Terranova et al., 2005) and sarizotan (Danysz et al., 2015) suggest efficacy in animal models of ADHD. It is also relevant that a *HTR1A* rs10042486 polymorphism is associated with ADHD (Park et al., 2013). Indeed, buspirone may benefit ADHD management (Levin, 2015), though to a lesser extent than methylphenidate (Mohammadi et al., 2012).

4. Autism Spectrum Disorder. Preclinical studies reveal altered central 5-HT_{1A} receptor activity, in a rat valproate model of autism (Wang et al., 2013b) and BTBR mice(BTBR $T^{+}Itpr3^{tf}$/J mouse), which have a phenotype paralleling that of autism spectrum disorder, elevated [^{35}S]GTPγS binding is evident, corresponding to enhanced 5-HT_{1A} receptor functional activity that potentially contributes to poor social behavior (Gould et al., 2011). Clinical data are limited, but anti–5-HT_{1A} receptor antibodies have been identified in the blood of an autistic boy (Todd and Ciaranello, 1985). Furthermore, a *HTR1A* C-1019G polymorphism in autism may influence clinical outcomes (Egawa et al., 2012).

5. Respiratory Control. 5-HT_{1A} receptor agonists increased respiration in rats and cats (Edwards et al., 1990; Rose et al., 1995), and morphine-induced ventilatory depression was reduced by the 5-HT_{1A} receptor agonist repinotan (Guenther et al., 2010). Electrophysiological studies support a modulatory role of the 5-HT_{1A} receptor in the bursting activity of respiratory neurons (Onimaru et al., 1998), and 5-HT_{1A} receptors activate bronchioconstrictor vagal preganglionic neurons and phrenic nerve neurons (Bootle et al., 1998; Valic et al., 2008). These and other data have led to the suggestion that 5-HT_{1A} receptor agonists display potential to treat sleep apnea (Futuro-Neto et al., 1993; Khater-Boidin et al., 1996, 1999; Dando et al., 1998; Sahibzada et al., 2000) that may translate to the clinic given an evident reduction in apnea evoked by buspirone (Wilken et al., 1997). In addition, activation of 5-HT_{1A} receptors may be beneficial to reverse compromised respiration; for instance, in a transgenic mouse model of Rett syndrome that also models disordered breathing, (+)8-OH-DPAT and sarizotan reduced the apneic frequency to restore the respiratory pattern (Abdala et al., 2010, 2014a,b; Levitt et al., 2013). Furthermore, the 5-HT_{1A} receptor–biased agonist, F15599, impacts apnea and respiration frequency in *MECP2*-null male and heterozygous female mice (Levitt et al., 2013). Clinical experiences investigating the 5-HT_{1A} receptor role in Rett syndrome are limited, but buspirone administered with fluoxetine reduced the frequency of hyperventilation and apneic attacks (Gokben et al., 2012).

6. Sexual Dysfunction. 5-HT_{1A} receptors may be a promising target in the treatment of sexual dysfunction. The 5-HT_{1A} receptor agonist flibanserin (which also possesses 5-HT_{2A} receptor antagonist and dopamine D_4 receptor partial agonist properties; Mendelson

and Gorzalka, 1986; Borsini et al., 2002; Heusler et al., 2009; Stahl, 2015) is a treatment of female hypoactive sexual desire disorder (Clayton et al., 2010; Jayne et al., 2012; Thorp et al., 2012; Katz et al., 2013) and is the culmination of research indicating a role for 5-HT_{1A} receptors in sexual function (e.g., Mendelson and Gorzalka, 1986; Olivier et al., 2011; Aubert et al., 2012; Gelez et al., 2013; Snoeren et al., 2014a,b), although its clinical effects are likely not exclusively related to actions at 5-HT_{1A} receptors (Allers et al., 2010; Stahl et al., 2011; Stahl, 2015). However, inclusion of 5-HT_{1A} agonist actions in the profile of activity of psychotropic drugs has been reasoned to potentially alleviate the sexual dysfunction seen in some patients treated with antidepressant or antipsychotic agents.

7. Food Intake and Eating Disorders. The role of 5-HT in modulating food intake and satiety has been investigated extensively (Blundell et al., 1995; Halford et al., 2007). Early studies demonstrated 5-HT_{1A} receptor activation induces hyperphagia, suggesting agonists may help treat patients with eating disorders such as bulimia and/or anorexia nervosa (Dourish et al., 1987). In vivo imaging studies suggest 5-HT_{1A} receptor binding increases in cortical and limbic structures of the brain of patients with anorexia and/or bulimia, consistent with a potential role in anxiety, behavioral inhibition, and body ideation (Kaye et al., 2005; Bailer et al., 2007, 2011; Galusca et al., 2008; Bailer and Kaye, 2011). Although clinical pharmacology studies are limited, and restricted to case studies, the partial agonist tandospirone improved the weight gain of patients with anorexia nervosa (restricting and binge-eating/purging subtypes) and also improved scores on the Eating Disorder Examination Questionnaire following treatment of up to 6 months (Okita et al., 2013). The mechanistic basis for this may involve control of mood: the anxiolytic effects of 5-HT_{1A} receptor agonists are likely to be beneficial (Crow and Mitchell, 1994) and potentially contribute to treatment outcome.

8. Aggressive Behavior. 5-HT_{1A} receptor activation appears to reduce aggressive behavior in preclinical and clinical (buspirone) settings (Olivier and Mos, 1992; Bell and Hobson, 1994; Takahashi et al., 2012) with animal models, indicating impact at the level of the dorsal raphe, and hence a reduction in 5-HT neurotransmission, may underlie the response (Mos et al., 1993). This is supported by results generated with S15535, a preferential autoreceptor agonist and, possibly, via blockade of hypersensitive postsynaptic 5-HT_{1A} heteroreceptors (Millan et al., 1997; de Boer et al., 2000). Indeed, elevated postsynaptic 5-HT_{1A} heteroceptors in the forebrain are associated with aggressive behavior (Korte et al., 1996), although direct administration of F15599 into ventral orbital PFC reduces aggression in male mice (Stein et al., 2013).

9. Neuroplasticity and Neuroprotection. 5-HT_{1A} receptor agonists evoke neurogenesis and synaptogenesis in the adult hippocampus, thereby improving cognitive performance in this structure that is important for mnemonic function (Mogha et al., 2012; Vines et al., 2012; Schreiber and Newman-Tancredi, 2014). Moreover, 5-HT_{1A} receptor stimulation can lead to long-term potentiation or depression (Meunier et al., 2013) with consequent elevated BDNF expression to influence neurogenesis (Luoni et al., 2013; Quesseveur et al., 2013).

In addition to the effects of 5-HT_{1A} receptor agonists on neuroplasticity, targeting this receptor may also have a beneficial role in neuroprotection. Indeed, there is considerable data supporting this assertion: repinotan reduced staurosporine-induced apoptosis (Suchanek et al., 1998), and 8-OH-DPAT reduced the impact of excitotoxic doses of NMDA in vivo (Oosterink et al., 1998) and, further, may protect neurons via protective effects of astrocytes; conversely, 5-HT_{1A} receptor antagonism by WAY100635 increased damage (Ramos et al., 2004). Similarly, the selective 5-HT_{1A} receptor agonist F13714 and the antipsychotic drugs clozapine, ziprasidone, and aripiprazole attenuated kainic acid–induced lesion volume in the striatum—effects that were reversed by WAY100635 (Cosi et al., 2005).

In models of Parkinson disease, 5-HT_{1A} receptor agonists may slow neuronal damage (Bezard et al., 2006) and limit astrogliosis (Miyazaki et al., 2013). In the experimental autoimmune encephalopathy model of multiple sclerosis and in vitro cell-based models, the efficacy of a novel arylpiperazine D_2/5-HT_{1A} receptor ligand suggested this was due to combined action of the compound to limit inflammation and neuroprotective actions (Popovic et al., 2015), and buspirone appears to exert some efficacy against apneusis in multiple sclerosis (O'Sullivan et al., 2008). Interestingly, repinotan was developed for activity in ischemic stroke and traumatic brain injury (Lutsep, 2002; Berends et al., 2005; Mauler and Horváth, 2005; Guenther et al., 2010), therapeutic areas that are historically very difficult for drug development. However, repinotan failed to show efficacy in acute ischemic stroke, and its development was discontinued (Teal et al., 2009).

III. 5-HT_{1B} Receptors

A. Introduction

The 5-HT_{1B} receptor and its counterpart the 5-HT_{1D} receptor have experienced a complex and debated history (Fig. 3) that is explained here. The two receptors are clearly closely related and result probably from gene duplication, which explains that in most species, their pharmacological profiles are almost indistinguishable (however, this is less evident in some species such as rat, mouse, hamster, or opossum; see below). In addition, 1) expression levels of the 5-HT_{1D} receptor are very low compared with those of the 5-HT_{1B} receptor, 2) the two receptors tend to be expressed together in many brain regions (although not in the periphery; Fig. 4), and 3) 5-HT_{1B} and 5-HT_{1D} receptors are coexpressed and

may form heterodimers in certain brain cells. In essence, the 5-HT_{1B} receptor is predominant, and, in the absence of selective compounds, it is very challenging to identify a separate population of 5-HT_{1D} receptors in the brain. Except in rodents, hamster, and opossum, in which both receptors display somewhat different pharmacological profiles, the 5-HT_{1B} receptor is still largely predominant in terms of expression and function.

The 5-HT_{1B} receptor was originally defined according to operational and transductional criteria, and it was initially thought to be a rodent-specific receptor [for references, see Hoyer et al. (1994)]. In the 1970s, Peroutka and Snyder (1979) and others postulated that whereas [^{3}H]5-HT labeled 5-HT_1 binding sites, [^{3}H]spiperone (and later [^{3}H]ketanserin) labeled 5-HT_2 binding sites, and [^{3}H]LSD labeled both 5-HT_1 and 5-HT_2 binding sites. In 1981, Nelson and colleagues (Pedigo et al., 1981) proposed that 5-HT_1 binding sites were a heterogeneous population, as [^{3}H]5-HT was displaced biphasically by spiperone; accordingly, the high affinity site for spiperone was called 5-HT_{1A}, and the low affinity was 5-HT_{1B}. Middlemiss et al. (1977) had reported earlier that certain indole β-blockers displayed high affinity for some 5-HT receptors. In 1982/1983, a breakthrough was reached when Hjorth et al. (1982) and Middlemiss and Fozard (1983) described 8-OH-DPAT as a selective 5-HT_{1A} ligand. Furthermore, Gozlan et al. (1983) reported the selective labeling of 5-HT_{1A} sites using [^{3}H]8-OH-DPAT. This allowed a clear definition of the 5-HT_{1A} pharmacological profile and, by extension, of the features of non–5-HT_{1A} sites [see Pazos et al. (1984a,b); Hoyer et al. (1985a,b)]. Thus, Palacios and Hoyer and colleagues (Hoyer et al, 1985b) at Sandoz in Basel characterized [^{3}H]mesulergine binding in the choroid plexus (Pazos et al., 1984a), which 5-HT competed for with high affinity, but the relatively low affinity of ketanserin and spiperone suggested a 5-HT_1 receptor pharmacology. The features of [^{3}H]mesulergine-labeled sites were different from classic 5-HT_2 binding sites labeled with, for example, [^{3}H]ketanserin. The novel [^{3}H]mesulergine-labeled binding site was named 5-HT_{1C} (now 5-HT_{2C}). Indeed, [^{3}H]mesulergine binding was also markedly different from 5-HT_{1B} binding as evidenced in radioligand binding and autoradiographic studies (Hoyer et al., 1985a,b, 1986a,b; Pazos and Palacios, 1985; Pazos et al., 1985, 1987a,b). More specifically in rodents, 5-HT_{1B} binding sites were characterized extensively with the iodinated version of cyanopindolol, [^{125}]ICYP (Engel et al., 1981), a potent β-blocker with high affinity for 5-HT_{1B} binding sites. These sites displayed high affinity for 5-HT, 5-carboxamidotryptamine (5-CT), some β-blockers, some ergolines, lysergic acid diethylamide (LSD), and RU24969 (Hoyer et al., 1985a, 1986a; Engel et al., 1986). Species differences in receptor pharmacology soon became evident with [^{3}H]mesulergine, which had different binding profiles in rodents, pigs, and humans;

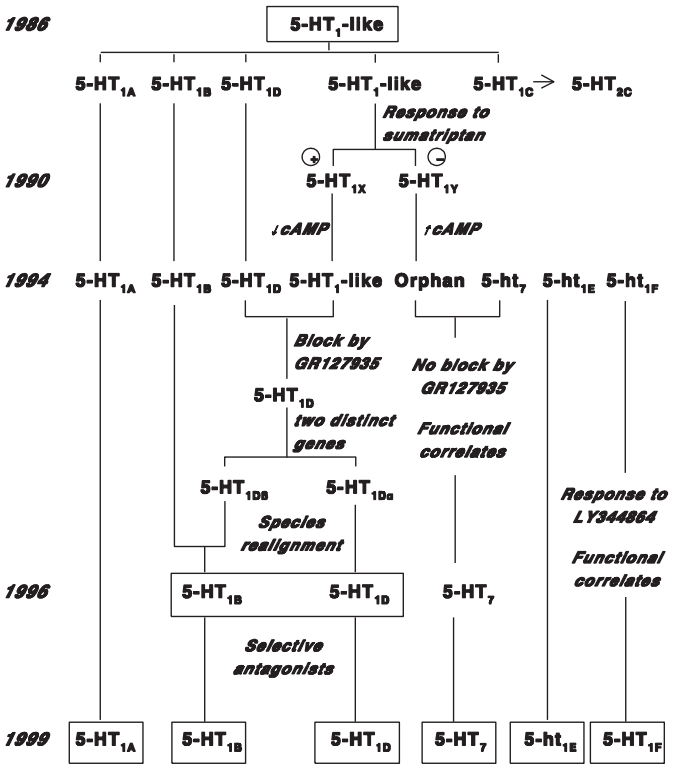

Fig. 3. The evolution of "5-HT_1–like" receptors into the different sumatriptan-sensitive 5-HT_1 receptor subtypes and the sumatriptan-insensitive 5-HT_7 receptor. Modified from Saxena et al. (1998). For references, see Hartig et al. (1996), Hoyer and Martin (1997), Villalón et al. (1997a), and Villalón and Centurión (2007).

this pattern would repeat itself with a number of 5-HT receptors, most prominently with the 5-HT_{1B} receptor (Hoyer et al., 1988; Waeber et al., 1988a,b). The Sandoz group used rat, mouse, hamster, rabbit, guinea pig, cat, dog, bovine, human, and more atypical for research species such as pigeons, opossum, and trout (e.g., Waeber et al., 1988a,b, 1989a,b) to investigate the pharmacology of 5-$HT_{1A,\ 1B,\ 1C}$, and 5-HT_2 receptor–binding sites. In addition, the pharmacology, transduction, and distribution of non–5-$HT_{1A/1B/1C}$ receptor binding sites, identified initially in calf and human brain and then most other species investigated, was termed 5-HT_{1D} receptor binding sites (Hoyer and Schoeffter, 1988; Schoeffter et al., 1988; Waeber et al., 1988a,b; Hoyer et al., 1988). Although the pharmacology of 5-HT_{1B} and 5-HT_{1D} binding sites/receptors displayed some distinct differences, their distribution pattern in brain was similar (if not overlapping), and they shared transductional and functional responses (Hoyer and Schoeffter, 1988; Schoeffter and Hoyer, 1989a, 1990). Therefore, rodent "5-HT_{1B}" and nonrodent "5-HT_{1D}" receptors were proposed initially to represent species homologs (Hoyer and Middlemiss, 1989), a view that was unequivocally confirmed when genetic and structural information became available with the cloning of these receptors (Voigt et al., 1991; Adham et al., 1992; Hamblin et al., 1992a,b; Hartig et al., 1992; Levy et al., 1992b; Maroteaux et al., 1992; Mochizuki et al., 1992).

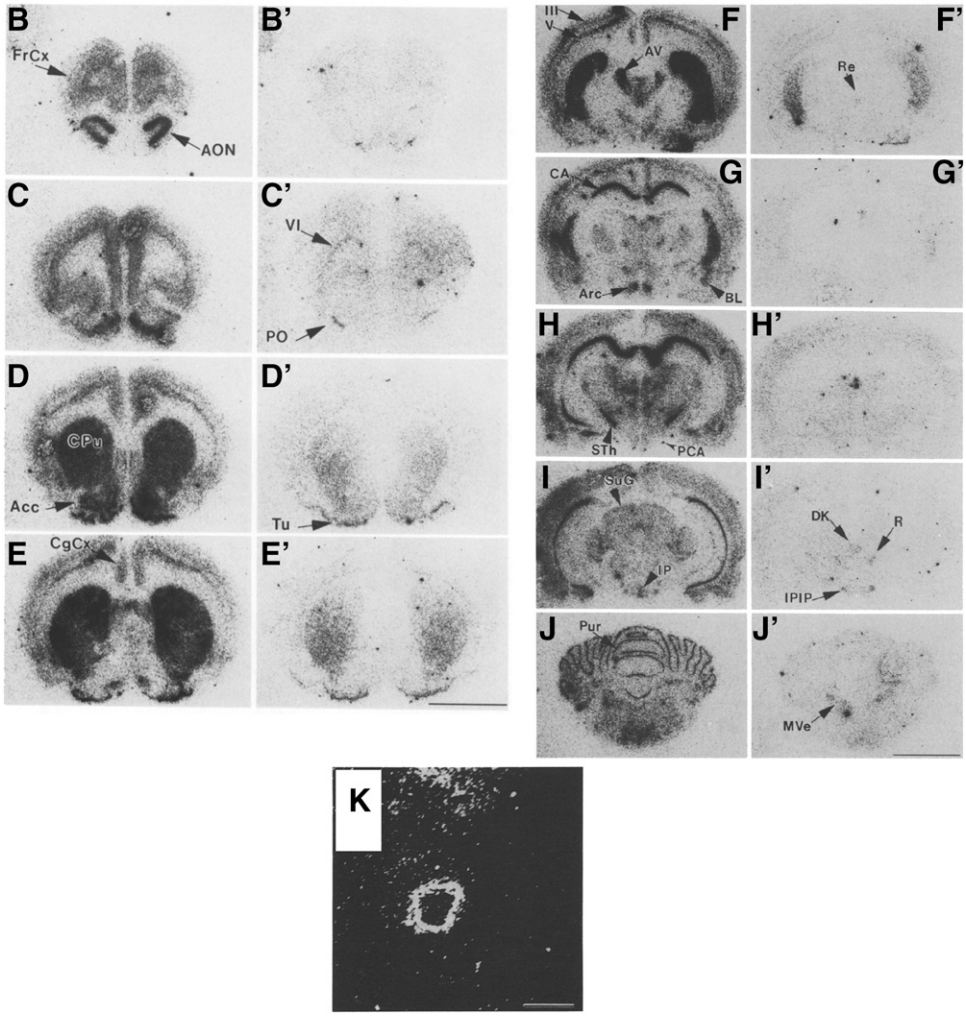

Fig. 4. In situ hybridization detection of 5-HT_{1B} and 5-HT_{1D} receptor mRNA in rat brain (and 5-HT_{1B} receptor mRNA in the posterior communicating artery [reverse autoradiogram; K]). 5-HT_{1B} (B–K) and 5-HT_{1D} (B'–J') receptor mRNA. Ace, nucleus accumbens; AON, anterior olfactory nucleus; Arc, arcuate hypothalamic nucleus; AV, anteroventral thalamic nucleus; BL, basolateral amygdaloid nucleus; CA1, CA1 region of the hippocampus; CgCx, cingulate cortex; CPu, caudate putamen; DK, nucleus of Darkschewitsch; FrCx [layer VI], frontal cortex; IP, interpeduncular nucleus; IPIP, inner posterior subnucleus of the interpeduncular nucleus; layers III and V, parietal motor cortex; MVe, medial vestibular nucleus; PCA, posterior communicating artery; PO, primary olfactory cortex; Pur, Purkinje cells of the cerebellum; R, red nucleus; Re, reuniens nucleus; STh, subthalamic nucleus; SuG, superficial gray layer of the superior colliculus; Tu, olfactory tubercle. Scale bar, 5 mm (except K, where it is 0.5 mm). Adapted from Bruinvels et al. (1994a) (with permission).

However, matters were further complicated when Weinshank et al. (1992) identified two structurally distinct genes encoding human 5-HT_1 receptors with, at the time, almost overlapping pharmacological profiles, both resembling the 5-HT_{1D} receptor. Earlier on, a canine "orphan" clone called RDC4 (later named 5-$HT_{1D\alpha}$) had been reported to display a 5-HT_{1D}–like pharmacological profile (Libert et al., 1990; Hamblin and Metcalf, 1991; Maenhaut et al., 1991; Zgombick et al., 1991). A human receptor, initially called S12, was cloned independently and differed in sequence from the canine RDC4, yet it displayed 5-HT_{1D}–like pharmacology (Levy et al., 1992b). Since the operational profiles of these two new receptors were mostly indistinguishable, they were called 5-$HT_{1D\alpha}$ (canine RDC4 and species homologs) and 5-$HT_{1D\beta}$ receptors (human S12 and species homologs). It soon became evident, however, that in spite of some fundamental differences in their pharmacological profiles (see below), the 5-$HT_{1D\beta}$ receptor was a human homolog of the rodent 5-HT_{1B} receptor (displaying 96% overall sequence homology; Adham et al., 1992). The subsequent identification of the 5-$HT_{1D\alpha}$ gene in rats confirmed that 5-HT_{1B} and 5-HT_{1D} receptors represent just two different receptor classes (Hartig et al., 1992), which prompted a realignment of 5-HT receptor nomenclature to recognize primacy (preeminence) of the human genome (Hartig et al., 1996). As a result, the 5-$HT_{1D\beta}$ receptor was renamed 5-HT_{1B} (subsuming the rodent 5-HT_{1B} receptor), whereas the 5-$HT_{1D\alpha}$ nomenclature was abandoned for 5-HT_{1D} in recognition of the fact that this gene product encodes the 5-HT_{1D} receptor (see Fig. 3; Hartig et al., 1996). This nomenclature for 5-HT_{1B} and 5-HT_{1D} receptors has been used since 1996 and remains to date.

B. Pharmacology

The 5-HT_1–like receptor mediating smooth muscle contraction and inhibition of noradrenaline release showed close similarities to the 5-HT_{1B} and/or 5-HT_{1D} receptors; however, the lack of selective ligands at these receptors made it difficult to distinguish these receptors with confidence, hampering research for quite some time (Hoyer, 1988a; Hoyer et al., 1994). Clitherow et al. (1994) reported the properties of several compounds, including a piperazinylbenzanilide derivative, GR127935, which shows a high affinity for and selective antagonist activity at 5-$HT_{1B/1D}$ receptors. But more importantly, the subsequent identification of potent and relatively selective antagonists at either the 5-HT_{1B} (SB224289; Hagan et al., 1997; Gaster et al., 1998) or 5-HT_{1D} (BRL15572; Price et al., 1997) receptors allowed responses to be attributed to either 5-HT_{1B} or 5-HT_{1D} receptors; for example, the sumatriptan-induced contraction of vascular smooth muscle was mediated via the 5-HT_{1B} receptor (e.g., De Vries et al., 1998, 1999; Verheggen et al., 1998, 2004).

Despite the 96% amino acid sequence homology in the transmembrane regions (Adham et al., 1992), the rodent 5-HT_{1B} receptor displays a distinct pharmacology compared with the 5-HT_{1B} receptor in other species (Hartig et al., 1996). The differences in the pharmacology of these species homologs are largely attributed to the mutation of a single amino acid in the transmembrane spanning region Asp^{123} to Arg^{123} (Adham et al., 1994a). Thus, CP93129 is a selective agonist at the rodent 5-HT_{1B} receptor, whereas some β-adrenoceptor antagonists, such as cyanopindolol, (−)pindolol, and (−)propranolol, are selective antagonists at the rodent 5-HT_{1B} receptor but not in other species. Unfortunately, no selective agonist is thus far available for the nonrodent 5-HT_{1B} receptor.

C. Receptor Structure and Transduction

The 5-HT_{1B} receptor gene is intronless, encoding for a 386-amino-acid protein in rat and mouse and 390-amino-acid protein in humans that displays the typical structure of a seven-transmembrane–spanning GPCR. The human, mouse, and rat 5-HT_{1B} receptor genes are located on chromosomes 6q13, 9E1, and 8q31, respectively. The rat receptor has 96% homology in the TMR with the human receptor, but the rat and mouse receptor (Voigt et al., 1991; Adham et al., 1992; Maroteaux et al., 1992) exhibit the typical 5-HT_{1B} receptor operational profile in contrast to the human receptor, which is close to the 5-HT_{1D} receptor operational profile (Levy et al., 1992b; Weinshank et al., 1992).

The 5-HT_{1B} receptor couples negatively to adenylyl cyclase (Bouhelal et al., 1988; Hoyer and Schoeffter, 1988, 1991; Adham et al., 1992; Levy et al., 1992b; Maroteaux et al., 1992). Native 5-HT_{1B} receptors expressed in opossum kidney cells also mediate elevation of intracellular calcium (Zgombick and Branchek, 1998).

It is noteworthy that 5-HT_{1B} (and 5-HT_{2B}) receptors have been crystallized (Wang et al., 2013; Wacker et al., 2013; McCorvy and Roth, 2015; see section *XVI. A. 5-HT GPCRs*), which greatly increases knowledge of the structure pharmacology of the receptor. Indeed, the conformation of a number of agonists is different when bound to 5-HT_{1B} or 5-HT_{2B} receptors, in spite of very similar orthosteric binding sites (Wacker et al., 2013; Wang et al., 2013; McCorvy and Roth, 2015). Sumatriptan and a range of other triptans fit well into the orthosteric pocket of the human 5-HT_{1B} receptor (in contrast to the 5-HT_{2B} receptor), thus confirming the high affinity and potency reported for the triptans at 5-HT_{1B} (and 5-HT_{1D}) receptors. Some ergolines [LSD, metergoline, dihydroergotamine (DHE), ergotamine] bind to an accessory, possibly allosteric, site, which is located outside of the orthosteric pocket. It has been proposed that a short peptide, 5-HT-moduline, is a negative allosteric modulator of both 5-HT_{1B} and 5-HT_{1D} receptors (Rousselle et al., 1998). Research concerning this peptide appears to have waned in recent years; the interested reader is directed to previous reviews on the subject (Fillion, 2000; Moret et al., 2003).

D. Distribution and Function

Autoradiographic studies performed in various species showed that both 5-HT_{1A} and 5-HT_{1C} (now named 5-HT_{2C}) receptor binding was evident, in addition to 5-HT_2 receptor binding. However, what was then called 5-HT_{1B} binding site was apparently absent in pig, calf, and human brain in contrast to rodent brain. This observation was extended to the guinea pig and then to an increasing number of other species (Hoyer at al., 1988; Waeber et al., 1988a,b; Hoyer and Middlemiss, 1989). Eventually, it was found that only rat, mouse, hamster, and opossum had a 5-HT_1 receptor with a classic 5-HT_{1B} profile [see Hoyer et al. (1985a,b)]. By contrast, other species expressed what was called 5-HT_{1D} receptors in the brain (e.g., guinea pig, bovine, dog, rabbit, monkey, and humans) (see Waeber et al., 1988a, 1989a,b; Hoyer and Schoeffter, 1991; Hoyer et al., 1992). It was subsequently shown that [^{3}H]sumatriptan and a number of other triptans label both 5-HT_{1B} and 5-HT_{1D} sites. However, they may also label 5-HT_{1F} sites (Waeber and Moskowitz, 1995b). It also became evident when using selective antagonists that both 5-HT_{1B} and 5-HT_{1D} receptors could be detected in a single species (Bruinvels et al., 1993a,b, 1994a; Doménech et al., 1997; Bonaventure et al., 1997; Napier et al., 1999; Varnäs et al., 2001), but 5-HT_{1D} receptor levels were minor when compared with the 5-HT_{1B} receptor.

An elegant study demonstrated the rat brain autoreceptors mediating inhibition of 5-HT release displayed the pharmacology of the 5-HT_{1B} receptor (Engel et al., 1986). In various other species, including humans, inhibitory autoreceptors displayed 5-HT_{1D} receptor pharmacology (Schlicker et al., 1989). 5-HT_{1B} receptors were also reported to mediate inhibition of GABA, cholinergic, and glutamatergic neurotransmission (Maura and Raiteri, 1986; Johnson et al., 1992; Singer et al., 1996;

Chadha et al., 2000; Morikawa et al., 2000). The 5-HT_{1B} receptor is highly concentrated in the substantia nigra (SN) and was shown to be negatively coupled to adenylyl cyclase activity (Bouhelal et al., 1988; Hoyer and Schoeffter, 1988, 1991).

Both 5-HT_{1B} and 5-HT_{1D} receptors have a neuronal localization (Waeber et al., 1990a,b; Bruinvels et al., 1991, 1992a,b, 1993a,b, 1994a,b; Sari et al., 1999), including in the trigeminal ganglia (Bruinvels et al., 1992a, 1994a,b; Hou et al., 2001; Ma, 2001; Potrebic et al., 2003). There is also evidence that both receptors colocalize and may form heterodimers (Xie et al., 1999; Ma, 2001).

Evidence from radioligand binding experiments using 5-HT neuronal lesions is equivocal regarding the location of the rat 5-HT_{1B} receptor, with some studies finding that the lesion causes an upregulation of 5-HT_{1B} binding sites and others finding a downregulation in the same areas. However, it is now clear that, like the 5-HT_{1A} receptor, the 5-HT_{1B} receptor functions as a presynaptic autoreceptor (see also section *XVIII. 5-HT Receptors and the Brain*). In situ hybridization studies have located mRNA encoding the 5-HT_{1B} receptor in the dorsal and median raphe nuclei (Bruinvels et al., 1994a). Furthermore, 5-HT_{1B} receptor mRNA in the raphe nuclei is markedly reduced by a 5-HT neuronal lesion. Together, these data suggest that 5-HT_{1B} receptors are located both presynaptically (inhibitory autoreceptor) and postsynaptically (heteroreceptor) relative to 5-HT neurons [see Waeber et al. (1990b)]; as an example of the latter, 5-HT_{1B} heteroreceptors inhibit CGRP release from sensory perivascular nerves in the rat systemic vasculature (González-Hernández et al., 2010).

5-HT_{1B} receptors are also located on cerebral arteries and other vascular tissues mediating direct vasoconstriction [see Villalón et al. (2003) and Villalón and Centurión (2007)]. Furthermore, it seems that the receptor may be "silent" in a number of vascular preparations, becoming responsive in conditions such as atherosclerosis or when costimulated with "priming" factors (Sahin Erdemli et al., 1991; Kaumann et al., 1993, 1994). Other peripheral effects have also been described, such as 1) inhibition of noradrenaline release from sympathetic nerves in vena cava (Göthert et al., 1986) and systemic vasculature (Villalón et al., 1998) and 2) inhibition of plasma extravasation produced by trigeminal ganglion stimulation (Buzzi and Moskowitz., 1991). 5-HT_{1B} receptors also mediate vasoconstriction in the rat caudal arteries (Craig and Martin, 1993) and the canine external carotid circulation (De Vries et al., 1998) or guinea pig iliac artery (Sahin Erdemli et al., 1991), although endothelium-mediated relaxation has also been reported (Schoeffter and Hoyer, 1989, 1990). Interestingly 5-HT_{1B} receptor mRNA is more abundant within vascular smooth muscle cells compared with 5-HT_{1D} receptor mRNA (Bouchelet et al., 1996; Sgard et al., 1996). The latter was reinforced by evident 5-HT_{1B} but not 5-HT_{1D} receptor immunoreactivity in cranial blood vessels (Longmore et al., 1997). Consistent with these findings, the subsequent advent of the potent and relatively selective antagonists at either the 5-HT_{1B} (SB224289; Hagan et al., 1997; Gaster et al., 1998) or 5-HT_{1D} (BRL15572; Price et al., 1997) receptors made it possible to demonstrate that the 5-HT_{1B}, but not the 5-HT_{1D}, receptor mediates the sumatriptan-induced contraction of vascular smooth muscle (e.g., De Vries et al., 1998, 1999; Verheggen et al., 1998), 2004.

E. Radioligand Binding

Autoradiographic studies using [^{3}H]-5-HT (in the presence of 8-OH-DPAT), [^{125I}]ICYP (in the presence of isoprenaline), or [^{125}I]–carboxymethylglycyl iodotyrosinamide demonstrated a high density of 5-HT_{1B} sites in the rat basal ganglia (particularly the substantia nigra, globus pallidus, ventral pallidum, and entopeduncular nucleus) but also in many other regions (Hoyer, 1988; Palacios et al, 1992; Hoyer et al., 1994; Mengod et al., 2010). The discrimination of 5-HT_{1B} and 5-HT_{1D} receptors in both rodent and nonrodent species has become more straightforward with the availability of a new 5-$HT_{1B/1D}$ radioligand, namely, [^{3}H]-GR-125743 (Doménech et al., 1997; Varnäs et al., 2001), or various triptans (Leysen et al., 1996; Bonaventure et al., 1997; Napier et al., 1999) as well as cold ligands, which discriminate 5-HT_{1B} and 5-HT_{1D} receptors (Price et al., 1997; Middlemiss et al., 1999).

IV. 5-HT_{1D} Receptors

A. Introduction

To recap, following the cloning of 5-$HT_{1D\alpha}$ and 5-$HT_{1D\beta}$ receptor genes in various species, 5-$HT_{1D\alpha}$ was renamed the 5-HT_{1D}, and 5-$HT_{1D\beta}$ became the 5-HT_{1B} receptor, keeping in mind that 5-HT_{1D} expression levels are generally low compared with the 5-HT_{1B} receptor (see section *III. 5-HT_{1B} Receptors* for more detail).

B. Pharmacology

As noted in *III. 5-HT_{1B} Receptors*, the pharmacological distinction of 5-HT_{1B} from 5-HT_{1D} receptors was a challenge until the advent of selective and silent antagonists (devoid of intrinsic activity) for 5-HT_{1B} and 5-HT_{1D} receptors (Hagan et al., 1997).

A series of isochroman-6-carboxamide derivatives, including PNU109291 (Ennis et al., 1998), PNU142633 (McCall, 1997; McCall et al., 2002), and L775606 (MacLeod et al., 1997), have been reported to be selective 5-HT_{1D} receptor agonists, although they display low intrinsic efficacy at primate 5-HT_{1D} receptors in GTPγ^{35}S binding assays (Pregenzer et al., 1999).

The 5-HT_{1D} receptor is potently antagonized by the 5-$HT_{1B/1D}$ receptor antagonist GR127935 (Clitherow et al., 1994; Skingle et al., 1996) and by the selective 5-HT_{1D} receptor antagonist BRL15572 (Price et al., 1997). Additionally, some 5-HT_2 receptor antagonists (e.g.,

ketanserin and ritanserin) can discriminate the 5-HT_{1D} receptor from 5-HT_{1B} and 5-HT_{1F} receptors (Hoyer et al., 1994), although this is highly species-dependent (see Branchek et al., 1995; Zgombick et al., 1995, 1997). Sumatriptan and the second-generation triptans are potent agonists at the 5-HT_{1D} receptor (but also interact with 5-HT_{1B} and 5-HT_{1F} receptors; Villalón et al., 2003; Table 6). It has been demonstrated that the 5-HT_{1D} receptor is located preferentially on neuronal, rather than vascular, tissues (Ullmer et al., 1995; Sgard et al., 1996; Longmore et al., 1997).

Given the cardiovascular liabilities of triptans potentially via 5-HT_{1B} receptors expressed by vasculature (Nilsson et al., 1999a,b), which is not the case for 5-HT_{1D} receptors (Nilsson et al., 1999b), it was hypothesized that selective 5-HT_{1D} receptor agonists may treat migraine, with reduced cardiovascular side effects. Unfortunately, this has not translated to the clinic; the 5-HT_{1D} receptor agonist PNU-142633 was ineffective in the acute treatment of migraine (Gómez- Mancilla et al., 2001), although the intrinsic activity of this compound may complicate interpretation.

C. Receptor Structure and Transduction

The 5-HT_{1D} receptor gene, like the 5-HT_{1B} receptor gene, is intronless. The human 5-HT_{1D} receptor gene is located on chromosome 1p34.3-p36.3, codes for a 377-amino-acid protein, and possesses 63% overall structural homology with the 5-HT_{1B} receptor; the mouse and rat receptor genes are located on chromosomes 4D3 and 5q36 and code for 374-amino-acid proteins. These receptors are made of a single polypeptide chain that spans the membrane seven times, with the amino terminus being extracellular and the carboxyl terminus intracellular in the manner typical of GPCRs (Hamblin and Metcalf, 1991; Hamblin et al., 1992; Weinshank et al., 1992; Weydert et al., 1992). The receptor is negatively coupled to adenylyl cyclase activity (Weinshank et al., 1992).

D. Distribution and Function

The distribution of 5-HT_{1D} receptors is known but understood with less confidence because protein levels are low along with the relative difficulty of radioligands to discriminate this receptor from the 5-HT_{1B} receptor. Receptor autoradiographic studies in rat (CP93129-insensitive [^{125}I]–carboxymethylglycyl iodotyrosinamide binding) or human (ketanserin-insensitive [^{3}H]-sumatriptan binding) brain clearly indicate 5-HT_{1D} receptor site is expressed in the basal ganglia (globus pallidus, substantia nigra, and caudate putamen) and also the hippocampus and cortex (Pineyro et al., 1995; Hou et al., 2001; Potrebic et al., 2003; Mengod et al., 2010).

TABLE 6
pK_i values of sumatriptan and second-generation triptans at human (except when stated otherwise) 5-HT receptors

Receptor	Sumatriptan	Zolmitriptan	Naratriptan	Rizatriptan	Eletriptan	Almotriptan	Frovatriptan	F11356
5-HT_{1A}	6.4[a]	6.6[b]	7.6[c]	6.4[b]	7.4[b]	6.3[d]	7.3[e]	7.6[f]
	6.9[c]	6.5[g]	7.1[b]					
	6.0[b]		7.1 (rat)[h]					
5-HT_{1B}	7.8[a]	7.7[b]	8.1[b]	6.9[b]	8.0[b]	8.0[d]	8.6[e]	9.4[f]
	7.4[b]	8.3[g]	8.7[h]	7.7[i]				
	8.3[j]			8.1[k]				
5-HT_{1D}	8.5[a]	8.9[b]	8.4[b]	7.9[b]	8.9[b]	8.0[d]	8.4[e]	9.3[f]
	8.0[b]	9.2[g]	8.3[h]	8.6[k]				
5-ht_{1e}	5.8[a]	7.7[b]	7.7[b]	6.8[b]	7.3[b]		<6.0[e]	5.9[f]
	5.8[b]	8.0[k]						
	5.6[l]							
5-HT_{1F}	7.9[a]	7.5[b]	8.2[b]	6.8[b]	8.0[b]		7.0[e]	5.5[f]
	7.9[b]	7.2[g]						
	7.6[l]							
5-HT_{2A}	<5.5[b]	<5.5[b]	<5.5[b]	<5.5[b]	<5.5[b]		<5.3[e]	6.7[f]
5-HT_{2B}	6.9[m]	7.2[m]		6.6[m]				
5-HT_{2C}	<5.5[b]	<5.5[b]	<5.5[b]	<5.5[b]	<5.5[b]		<5.3[e]	
m5-HT_3	<5.5[b]	<5.5[b]	<5.5[b]	<5.5[b]	<5.5[b]		<6.0[e]	<5.0[f]
gp5-HT_4	<5.5[b]	<5.5[b]	<5.5[b]	<5.5[b]	<5.5[b]			5.7[f]
5-HT_{5A}	<5.5 (rat)[b]	6.4 (rat)[b]	5.5 (rat)[b]	5.3 (rat)[b]	5.8 (rat)[b]			6.1[f]
5-HT_6	<5.5[b]	<5.5[b]	<5.5[b]	<5.5[b]	6.3[b]			5.6[f]
5-HT_7	5.9[b]	7.0[b]	<5.5[b]	5.7[b]	6.7[b]	<6.5[d]	6.7[e]	6.4[f]

gp, guinea pig; m, Mouse.
[a]Data taken from Leysen et al. (1996).
[b]Data taken from Napier et al. (1999).
[c]Data taken from Newman-Tancredi et al. (1997b).
[d]Data taken from Bou et al. (1997).
[e]Data taken from Brown et al. (1998c).
[f]Data taken from John et al. (1999).
[g]Data taken from Martin (1997).
[h]Data taken from Connor et al. (1997).
[i]Data taken from Wurch et al. (1997).
[j]Data taken from Beer et al. (1998).
[k]Data taken from P. J. Pauwels, personal communication.
[l]Data taken from Adham et al. (1993).
[m]Data taken from P. Gupta, personal communication.

In situ hybridization experiments allow a greater confidence of gene expression, albeit not at the level of protein. Thus, 5-HT_{1D} mRNA is present in rat brain, including the caudate putamen, nucleus accumbens (NAc), olfactory cortex, dorsal raphe nucleus, and locus coeruleus (e.g., Bruinvels et al., 1994a; Mengod et al., 2010; Fig. 4). The mRNA shows low abundance in all regions and was undetectable in the globus pallidus, ventral pallidum, and substantia nigra where, as noted above, 5-HT_{1D} receptor sites appear to be present, which is perhaps indicative of the 5-HT_{1D} receptor being located predominantly on axon terminals of both 5-HT and non–5-HT neurons.

In the periphery, the presence of 5-HT_{1D} receptors is rather limited with evidence of presence in autonomic and trigeminal nerve terminals/ganglia (Molderings et al., 1996; Villalón et al., 1998).

The function of the 5-HT_{1D} receptor still remains, to some extent, enigmatic. There is little evidence supporting the role of the 5-HT_{1D} receptor in any pathology. The availability of suitable tools for investigation in vivo has limited the investigations into the importance of 5-HT_{1D} receptors; they have been identified as autoreceptors in the dorsal raphe (Pineyro et al., 1995) or terminal brain regions. Thus, given their autoreceptor activity, 5-HT_{1D} receptor antagonists may have antidepressant potential, and to maximize 5-HT release in terminal brain regions, 5-HT_{1D}, 5-HT_{1B}, and 5-HT_{1A} receptors must be blocked simultaneously.

Operationally, 5-HT_{1D} receptors mediate inhibition of noradrenaline release in human atrium. Additionally, the 5-HT_{1D} receptor seems to be involved in the inhibition of guinea pig dural plasma protein extravasation (Ennis et al., 1998) and the central trigeminal inhibitory effects by some antimigraine compounds (Mills and Martin, 1995; Cumberbatch et al., 1998; De Vries et al., 1999a,b; Villalón et al., 2003).

It has been proposed that the 5-HT_{1D} receptor modulates growth hormone release (Mota et al., 1995; Whale et al., 1999), although this requires clearer pharmacological verification.

V. 5-ht_{1e} Receptors

A. Introduction

There has been relatively limited research on the 5-ht_{1e} receptor, with an apparent lack of expression in rodents complicating preclinical studies. The lack of functional data concerning natively expressed 5-ht_{1e} receptors means by convention lower case appellation is still used for nomenclature.

With hindsight, the 5-ht_{1e} receptor was likely discovered by virtue of an atypical pharmacology of a [^{3}H]5-HT binding site in human frontal cortex (Leonhardt et al., 1989), which was sensitive to guanyl nucleotides, suggesting association with the GPCR family. The high affinity [^{3}H]5-HT displayed for the binding sites and the low affinity of drugs displaying affinity for the 5-HT_2 receptor (e.g., mesulergine) supported membership of the 5-HT_1 receptor family. However, the low affinity of 5-CT, the prototypical 5-HT_1 receptor agonist, and detailed pharmacological characterization of the new [^{3}H]5-HT binding site in human and bovine cortical homogenates highlighted that this site likely represented a further member of the 5-HT_1 family, and hence it was given that next available name: 5-HT_{1E} (Leonhardt et al., 1989; now reclassified as 5-ht_{1e} until a functional response in native tissue/cell preparation can be attributed).

B. Cloning and Distribution of 5-ht_{1e} Receptors

Soon after the 5-ht_{1e} receptor binding site was pharmacologically characterized by radioligand binding in human and bovine brain tissue, a human GPCR gene, termed S31, was cloned (Levy et al., 1992a; see h5-ht_{1e} in Fig. 5 for sequence) and assigned to human chromosome 6q14-q15 (Levy et al., 1994). When S31 was expressed in cell lines, the gene product was found to have pharmacological properties similar to the tissue-expressed 5-ht_{1e} receptor binding site, and the conclusion was that this gene encodes the protein for the receptor binding site discovered by Leonhardt et al. (1989) (McAllister et al., 1992; Zgombick et al., 1992; Gudermann et al., 1993). However, the 5-HT_{1F} receptor, discovered subsequent to these early reports on the 5-ht_{1e} receptor, shares a high degree of sequence homology with the 5-ht_{1e} receptor compared with other 5-HT receptors (see Fig. 5 for h5-ht_{1e} and h5-HT_{1F} amino acid sequence alignment) and bears a pharmacological profile very similar to the 5-ht_{1e} receptor (Adham et al., 1993a,b; Lovenberg et al., 1993b). A careful examination of the binding data presented in the original report on the 5-ht_{1e} receptor (Leonhardt et al., 1989) suggests the binding site identified in this report is likely a composite of both 5-ht_{1e} and 5-HT_{1F} receptor binding sites. Drugs that can discriminate between these receptor subtypes were not identified until after a number of studies were published that attempted to identify the distribution of 5-ht_{1e} receptors via radioligand binding and autoradiography methodologies (Miller and Teitler, 1992; Beer et al., 1993; Barone et al., 1994; Stanton et al., 1996; Fugelli et al., 1997). This resulted in reports that incorrectly attributed [^{3}H]5-HT radioligand binding to the 5-ht_{1e} receptor in both rat and mouse brain tissue, species that were later identified to lack the 5-ht_{1e} receptor gene (Bai et al., 2004). Even those reports that used tissue from species that do express a 5-ht_{1e} receptor gene (e.g., humans, monkeys, guinea pigs, and bovine) were, in hindsight, confounded by the labeling of 5-HT_{1F} receptors and thus need to be viewed as data that reflects a mixture of 5-ht_{1e} and 5-HT_{1F} receptor populations. Subsequent pharmacological isolation of 5-ht_{1e} receptors (Klein and Teitler, 2012) has shown the following pattern of expression of the

5-ht$_{1e}$ receptor binding sites: olfactory bulb = hippocampus > frontal cortex > hypothalamus = cerebellum > brainstem-thalamus = striatum (Fig. 6). Issues impacting the ability to define with confidence the distribution of the 5-ht$_{1e}$ receptor were to some extent overcome with the development of an antibody recognizing the 5-ht$_{1e}$ receptor protein (Klein and Teitler, 2012), allowing protein expression to be revealed in native tissue. Such immunohistochemical studies revealed that the 5-ht$_{1e}$ receptor immunoreactivity was expressed in the olfactory bulb (glomerula cells), whereas in the hippocampus, expression is limited to the dentate gyrus (Klein and Teitler, 2012). Interestingly, 5-ht$_{1e}$ receptor immunoreactivity was also expressed in cerebral arteries (guinea pig; Klein and Teitler, 2012).

C. Pharmacology

Bai et al. (2004) demonstrated that the rhesus monkey, pig, rabbit, and guinea pig express a homolog of the human 5-ht$_{1e}$ receptor gene. Because of the relative utility in preclinical models, the guinea pig 5-ht$_{1e}$ receptor (gp5-ht$_{1e}$) sequence was cloned for further study. The guinea pig homolog shares 88% nucleic acid and 95% amino acid sequence homology with the human 5-ht$_{1e}$ receptor. The pharmacological properties of the guinea pig recombinant 5-ht$_{1e}$ receptor correlate well with the human counterpart in terms of affinity (R^2 = 0.99) and potency (R^2 = 0.96), indicating a high degree of evolutionary conservation for the receptor.

Quantitative RT-PCR of guinea pig brain regions revealed high levels of gp5-ht$_{1e}$ receptor mRNA in the cortex, hippocampus, and olfactory bulb and moderate expression in some other regions, similar to the expression pattern in the human brain (Bai et al., 2004). Thus, the structural and pharmacological similarities of the human and guinea pig receptors, along with comparable patterns of expression in gross brain regions, lend a great deal of support to the guinea pig as a valid model to study the functionality of the h5-ht$_{1e}$ receptor.

Some attempts have been made to develop selective pharmacological tools for the h5-ht$_{1e}$ receptor (Dukat et al., 2004) but failed to identify 5-ht$_{1e}$ receptor ligands with affinities substantially higher than 5-HT. A relatively selective high-affinity 5-ht$_{1e}$ receptor ligand has been identified, BRL54443 (Brown et al., 1998); this drug displays similar affinities for the h5-ht$_{1e}$ and h5-HT$_{1F}$ receptors but, more usefully, at least 60-fold lower affinities for other 5-HT, dopamine, and adrenergic receptors. Few published reports exist regarding the pharmacology of this compound, and the reports of BRL54443 action in vivo have used species that do not express the 5-ht$_{1e}$ receptor (mice and rats; Adham et al., 1994; Brown et al., 1998; McKune and Watts, 2001; Watts et al., 2001; Hisadome et al., 2009; Granados-Soto et al., 2010).

A high throughput screening study conducted at the Scripps Research Institute's Molecular Screening Center in collaboration with Milt Teitler's laboratory, with the aim of identifying highly potent, selective agonists or antagonists for the 5-ht$_{1e}$ receptor, has been performed [PubChem BioAssay Database, AID (accession #): 567; 574; 613; 718; 726; 730]. Nearly 65,000 compounds from a broad range of structural classes were screened for agonist and antagonist properties at the h5-ht$_{1e}$ receptor and counter-screened at the 5-HT$_{1A}$ receptor as an assessment of selectivity. Though none of the compounds were highly selective for the h5-ht$_{1e}$ receptor, a number of high-potency agonists (EC_{50} low nanomolars) were identified that displayed some structural similarity to BRL54443. In a more recent study comparing 51 tryptamine-based compounds for affinities at the human 5-ht$_{1e}$ and 5-HT$_{1F}$ receptors, no drugs were identified that showed a significant preference for the 5-ht$_{1e}$ receptor over the

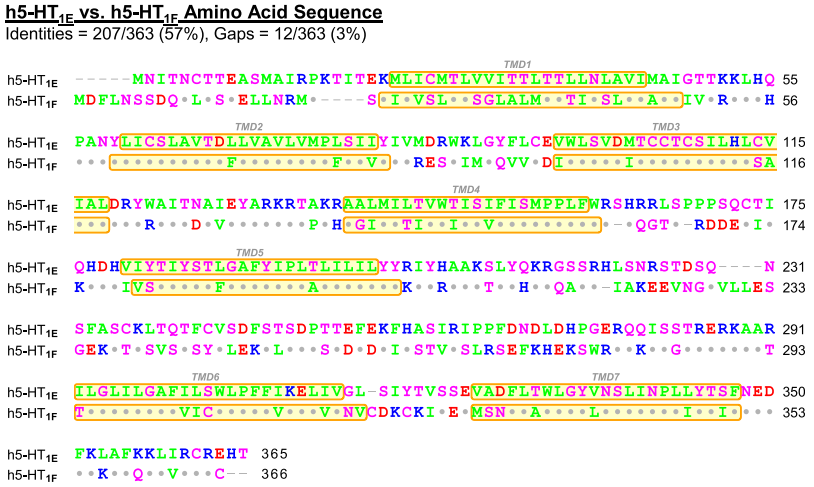

Fig. 5. Alignment of human 5-ht$_{1e}$ and 5-HT$_{1F}$ receptor amino acid sequences. Sequence homology is assessed by Basic Local Alignment Search Tool (BLAST, copyright National Library of Medicine) for protein sequences. Transmembrane domains (TMD 1–7) were determined previously (Bai et al., 2004) and highlighted by yellow rectangles. Nonpolar, uncharged polar, acidic polar, and basic polar amino acids are labeled by corresponding color.

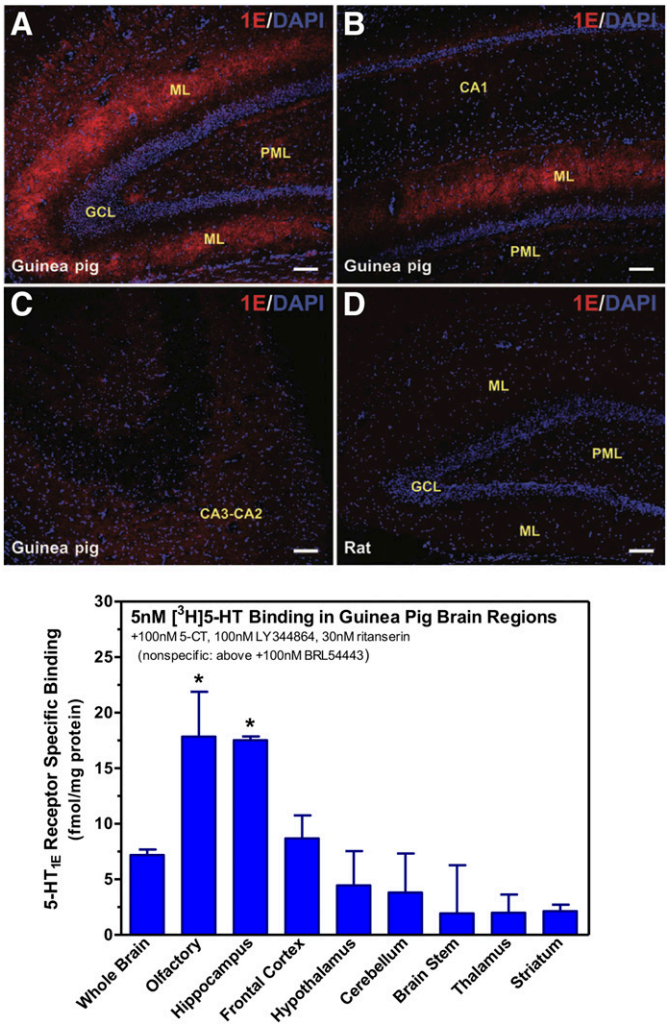

Fig. 6. 5-ht$_{1e}$ receptor expression in guinea pig and rat brain. The top image shows immunohistochemical staining of the 5-ht$_{1e}$ receptor in the hippocampus. (A) Coronal section of guinea pig hippocampus at the septal pole of the DG. (B) Coronal section of guinea pig hippocampus near the temporal pole of the DG. (C) Coronal section of rostral guinea pig hippocampus shows a lack of 5-ht$_{1e}$ receptor staining in the CA3-CA2 region. (D) Rat hippocampus (coronal section of DG septal pole) does not stain for 5-ht$_{1e}$ receptors. GCL, granular cell layer; ML, molecular layer; PML, polymorphic layer. Scale bars, 100 μm. The bottom image shows histogram of radioligand 5-ht$_{1e}$ receptor binding sites in homogenates of guinea pig brain. High levels of 5-ht$_{1e}$ receptor binding sites are detected in the hippocampus and olfactory bulb. *$P < 0.05$ compared with "whole-brain," one-way ANOVA with Dunnett's post-test. Data are the means ± S.E.M. of three independent experiments performed in triplicate. Adapted from Klein and Teitler (2012) (with permission).

5-HT$_{1F}$ receptor, again demonstrating the difficulties in attempting to identify 5-ht$_{1e}$ receptor–selective drugs (Klein et al., 2011).

D. Functions

Recombinant expression of the 5-ht$_{1e}$ receptor in cell lines demonstrates the coupling to $G_{i/o}$ signaling pathways (Levy et al., 1992a; Gudermann et al., 1993; Adham et al., 1994) although no signaling pathways have been identified in native tissues, and in the absence of a recognized functional response, the lower case appellation nomenclature is retained.

VI. 5-HT$_{1F}$ Receptors

A. Introduction

Although the first published reports for the 5-HT$_{1F}$ receptor occurred in 1992 and 1993 (Amlaiky et al., 1992; Adham et al., 1993b; Lovenberg et al., 1993), there is still only limited information about this receptor. Much of the current literature centers on possible roles for the 5-HT$_{1F}$ receptor in the treatment of migraine despite the 5-HT$_{1F}$ receptor displaying a broad distribution within the central nervous system (CNS), and it also appears to be expressed in the periphery.

B. Cloning and Structure

The 5-HT$_{1F}$ receptor was discovered as the result of homology cloning strategies. The first report of the cloning of the human 5-HT$_{1F}$ receptor was in a patent application by Synaptic Pharmaceuticals, Inc., (Weinshank et al. 1994, U.S. patent number 5,360,735, filed in 1992, issued in 1994). Amlaiky et al. (1992) reported the cloning of the mouse receptor (initially called 5-HT$_{1E\beta}$) that same year, followed by a peer-reviewed report on the human receptor (Adham et al., 1993b) and the cloning of both the rat and human versions (initially called 5-HT$_{1E\text{-like}}$) of the receptor in 1993 (Lovenberg et al., 1993) (Table 7).

The 5-HT$_{1F}$ receptor gene is intronless, coding for a GPCR of 366 amino acids that conform to the classic GPCR structure, and has been sequenced in a number of species (mouse, rat, guinea pig, pig, human; Amlaiky et al., 1992; Adham et al., 1993b, 1997; Lovenberg et al., 1993; Bhalla et al., 2002b). In mouse and rat, it appears that the 5-HT$_{1F}$ receptor is encoded by three different mRNA transcripts (Guptan et al., 1997), which differ in their 3′ untranslated regions.

C. Distribution

1. mRNA. The initial studies of 5-HT$_{1F}$ receptor distribution located 5-HT$_{1F}$ receptor mRNA, either by RT-PCR or in situ hybridization (Fig. 7; Table 8). 5-HT$_{1F}$ receptor mRNA has a rather broad distribution within the brain; the cerebral cortex shows a relatively dense band of expression within the internal layers (approximately layers IV–VI), and hippocampal areas CA1–CA3 display relatively high expression, as do the thalamus and striatum.

Documentation of the presence of 5-HT$_{1F}$ receptor mRNA in peripheral tissues is limited to a few single reports in different species, including human, bovine, pig, rat, and rabbit (Table 8). Overall, there is still a need for a systematic mapping of the peripheral distribution of 5-HT$_{1F}$ receptors. Peripheral blood vessels such as the coronary artery have been reported to express 5-HT$_{1F}$ message, although the findings in human coronary appear variable, with Ishida et al. (1999) finding none, Nilsson et al. (1999b) reporting a strong signal, and Bouchelet et al. (2000) reporting a weak signal in about 40% of patients. Bhalla et al.

(2002b) found a 5-HT_{1F} receptor mRNA signal in porcine coronary artery. Regardless, the 5-HT_{1F} receptor agonist LY334370 did not elicit contractions in human coronary artery (Nilsson et al., 1999b). 5-HT_{1F} receptor transcripts are also present in vascular preparations from the CNS (Table 8). However, when the brain microvessel preparations were treated to yield cultures of either smooth muscle or endothelial cells, no 5-HT_{1F} receptor mRNA was detected (Cohen et al., 1999).

In contrast to peripheral tissues, evidence for the presence of 5-HT_{1F} receptor mRNA in the peripheral nervous system is clear; thus, multiple studies in trigeminal ganglia and dorsal root ganglia (fresh or cultured) have identified 5-HT_{1F} receptor mRNA (Table 8).

2. Radioligand Binding. The first localization studies of 5-HT_{1F} receptor binding sites used [^{3}H]sumatriptan, which displays high affinity for 5-HT_{1F} receptors as well as 5-$HT_{1B/1D}$ receptors. This radioligand has been used to map the 5-HT_{1F} receptor in a variety of species (including "cold" competing ligands such as 5-CT or methiothepin prevents radiolabeling of 5-HT_{1B} and 5-HT_{1D} receptors; Waeber and Moskowitz, 1995b; Mengod et al., 1996; Scarr et al., 2004; Dean et al., 2006). Although [^{3}H]5-HT can be used to label heterologous expression of 5-HT_{1F} receptors in cultured cells (Adham et al., 1993b), lack of selectivity makes 5-HT_{1F} receptor localization studies with [^{3}H]5-HT challenging (Fugelli et al., 1997). A second useful radioligand to label the 5-HT_{1F} receptor is [^{3}H]LY334370, albeit in the presence of 5-HT_{1A} receptor ligands such as 8-OH-DPAT to prevent labeling of the latter receptor (Wainscott et al., 2005). It is probably noteworthy that with all 5-HT_{1F} receptor–labeling studies, the lack of a selective radioligand necessitating the use of "cold" blocking drugs to better isolate radioligand binding to the 5-HT_{1F} receptor may underestimate the reported levels of 5-HT_{1F} receptors, or conversely, the specific radioligand binding signal consists of a heterogenous population of sites that includes the 5-HT_{1F} receptor; either way, interpretation should be made with caution.

3. Immunoreactivity. Relatively few studies have used antibodies to localize 5-HT_{1F} receptors (Table 9). The antibody studies of Ma (2001) and Classey et al. (2010) are consistent with investigations showing 5-HT_{1F} receptor mRNA in trigeminal ganglia and dorsal root ganglia (Table 8). Classey et al. (2010) describe 5-HT_{1F} receptor–like immunoreactivity in dorsal root ganglia

TABLE 7
Chromosomal location of the 5-HT_{1F} receptor gene

The chromosomal location of 5-HT_{1F} receptor genes can be found in the following databases: http://www.ncbi.nlm.nih.gov/gene/?term=HTR1F%5Bsym%5D and http://www.guidetopharmacology.org/GRAC/ObjectDisplayForward?objectId=5.

Species	Locus	Name
Human[a]	3p12	HTR1F
Rat	11p12	Htr1f
Mouse	16 C1.3	Htr1f

[a]Maassen Vandenbrink et al. (1998).

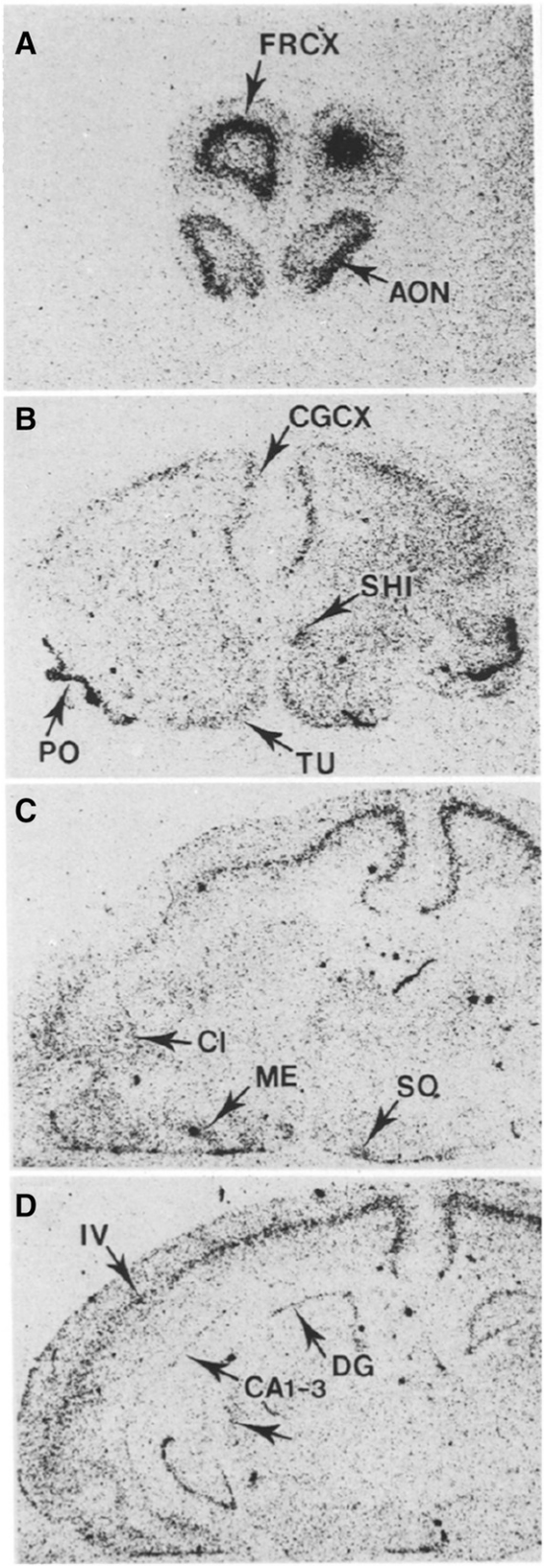

Fig. 7. In situ hybridization detection of 5-HT_{1F} receptor mRNA expression in guinea pig brain. (A) Frontal cortex (FRCX), anterior olfactory nucleus (AON). (B) Cingulate cortex (CGCX), septo-hippocampal nucleus (SHI), olfactory tubercle (TU), primary olfactory cortex (PO). (C) Claustrum (CL), medial amygdaloid nucleus (ME), supraoptic hypothalamic nucleus (SO). (D) Layer IV of the parietal motor cortex (IV), dentate gyrus (DG), and CA1-3 field (CA1-3) of the hippocampus are shown. Adapted from Bruinvels et al. (1994) (with permission).

TABLE 8
Localization of 5-HT_{1F} receptor mRNA

The most intensely labeled regions or areas of unique interest from the different references are listed in this table. See the original references for a more complete expression profile.

Tissue or Cells	Species	Method	Reference
Forebrain, hindbrain, spinal cord, cerebellum	Mouse	RT-PCR	Amlaiky et al., 1992
CA1, CA2, CA3 of the hippocampus	Mouse	ISHH	Amlaiky et al., 1992
Brain, uterus, mesentery	Human	RT-PCR	Adham et al., 1993a
Layer V of cerebral cortex (pyramidal cells), layer VI nonpyramidal cells, CA1-CA3 of the hippocampus (pyramidal cells), Dorsal raphe (large neurons), Dentate gyrus (granule cells)	Guinea pig	ISHH	Adham et al., 1993a
Cerebral cortex, striatum, hippocampus, thalamus, pons, hypothalamus, cerebellum	Rat	RT-PCR	Lovenberg et al., 1993
Olfactory, cingulate, frontal, and entorhinal cortex (especially layer V), hippocampus (CA1, CA2, CA3 and dentate gyrus)	Guinea Pig	ISHH	Bruinvels et al., 1994
Claustrum, cerebral cortex, thalamus, striatum	Guinea Pig	ISHH	Mengod et al., 1996
Trigeminal ganglion	Human	RT-PCR	Bouchelet et al., 1996
Cerebral blood vessels	Human	RT-PCR	Bouchelet et al., 1996
Trigeminal ganglion	Guinea pig	ISHH	Johnson et al., 1997
Dorsal root ganglia, trigeminal ganglion	Guinea pig	ISHH	Adham et al., 1997
Dorsal root ganglia	Human	RT-PCR	Pierce et al., 1997
Cerebral cortex, hippocampus, cerebellum, brain stem	Mouse	Northern blot	Guptan et al., 1997
Hippocampus, cerebral cortex	Rat	Northern blot	Guptan et al., 1997
Dorsal root ganglia (cultured)	Rat	RT-PCR	Chen et al., 1998
Neonatal and cultured astrocytes	Rat	RT-PCR	Hirst et al., 1998
Coronary arteries, atrium, ventricle, and epicardium	Human	RT-PCR	Nilsson et al., 1999b
Brain cortical microvessels and capillaries	Human	RT-PCR	Cohen et al., 1999
Astrocytes (cultured)	Human	RT-PCR	Cohen et al., 1999
Immune tissue: spleen, thymus, mitogen-activated spleen cells, and peripheral blood lymphocytes	Rat	RT-PCR	Stefulj et al., 2000
Lumbar dorsal root ganglia	Rat	RT-PCR	Wu et al., 2001
Cerebral cortex, trigeminal ganglion	Pig	RT-PCR	Bhalla et al., 2002
Coronary artery, pulmonary artery, saphenous vein	Pig	RT-PCR	Bhalla et al., 2002
Lumbar dorsal root ganglia	Rat	RT-PCR	Liu et al., 2005
Intestine (four separate regions)	Bovine	RT-PCR	Engel et al., 2006
Various forebrain areas of embryonic brain, including cerebral cortex, amygdala, globus pallidus, caudate putamen, hippocampus, dentate gyrus	Mouse	ISHH	Bonnin et al., 2006
Midbrain periaqueductal gray	Rat	RT-PCR	Jeong et al., 2008
Neonatal rat: cerebellum, cortex, cortical astrocytes; adult rat: cerebellum, cortex	Rat	RT-PCR	Osredkar and Kržan, 2009
Trigeminal ganglion, trigeminal nucleus caudalis	Rat	RT-PCR	Amrutkar et al., 2012
Renal proximal tubule cells	Rabbit	RT-PCR	Garrett et al., 2014
Heart, liver	Mouse	RT-PCR	Garrett et al., 2014

from all regions examined, including cervical, thoracic, and lumbar. Ma (2001) reported colocalization of 5-HT_{1F} receptor with glutamate-containing neurons of the trigeminal ganglion, and Ahn et al. (2009) showed colocalization of 5-HT_{1F} receptors with CGRP in neurons of rat vestibular nuclei. Garrett et al. (2014) provide a unique perspective on the 5-HT_{1F} receptor, showing the presence of both mRNA (Table 8) and protein (Table 9) in the rabbit renal proximal tubule.

D. Pharmacology

1. Agonists. The 5-HT_{1F} receptor, like the other members of the 5-HT_1 family, has high affinity for 5-HT itself. This characteristic has driven much of the structural work devoted to developing high-affinity, selective 5-HT_{1F} receptor agonists.

Compared with 5-HT_{1A}, 5-HT_{1B}, and 5-HT_{1D} receptors, the pharmacology described for 5-HT_{1F} receptors is rather sparse. Because of a potential link between 5-HT_{1F} receptor activation and the treatment of migraine (see below), most of the effort to develop selective compounds has focused on the development of orthosteric agonists. No selective orthosteric 5-HT_{1F} receptor antagonists have been reported. Likewise, there are no literature descriptions of allosteric 5-HT_{1F} receptor ligands.

Almost all of the structure activity relationship (SAR) work has come from the laboratories of Eli Lilly and Company, which had exclusive rights to the 5-HT_{1F} receptor through a collaborative agreement with the patent holder, Synaptic Pharmaceutical Corporation. Table 10 summarizes published named compounds showing selectivity for the 5-HT_{1F} receptor. Lasmiditan (COL-144, LY573144) and LY344864 display very good selectivity for the 5-HT_{1F} receptor relative to all other 5-HT receptors, as does LY334370, except that it is only about eight- to ninefold selective over the 5-HT_{1A} receptor. LY302148 was an early molecule that showed selectivity for the 5-HT_{1F} receptor relative to other 5-HT_1 subtypes. However, it also has high affinity for 5-HT_{2A} and 5-HT_{2B} receptors (Table 10) and is illustrative of the difficulty in identifying 5-HT receptor subtype selectivity in simple indole-based compounds.

Lasmiditan (COL-144, LY573144) is a departure from the other structures in that it contains no indole nucleus. The progression from a bicyclic aromatic nucleus (indole)

TABLE 9
Localization of 5-HT_{1F} receptor protein using antibodies

Tissue or Cells	Species	Method	Reference
Trigeminal ganglion neurons	Rat	Immunohistochemistry	Ma, 2001
Superior, lateral, spinal, and medial vestibular nuclei	Rat	Immunohistochemistry	Ahn et al., 2009
Neurons of trigeminal ganglia and dorsal root ganglia (C2, C5, T5, and L5 levels)	Rat	Immunohistochemistry	Classey et al., 2010
Renal proximal tubule cells	Rabbit	Immunoblot	Garrett et al., 2014

to the monocyclic nucleus of lasmiditan apparently involved some serendipity, as Zhang et al. (2015) describe how, in the process of producing a homolog of LY334370, a monocyclic intermediate was formed that had moderately good affinity for the 5-HT_{1F} receptor. Expanding an SAR around this finding, they discovered several compounds that had high affinity and good selectivity for the 5-HT_{1F} receptor (Zhang et al., 2015). Replacing the indole to eventually generate lasmiditan resulted in a highly selective 5-HT_{1F} receptor agonist.

Several additional studies have generated significant SAR for 5-HT_{1F} receptor agonists (Xu et al., 2001; Filla et al., 2003; Mathes et al., 2004; Zhang et al., 2004). The most potent and selective compounds are included in Table 11. These molecules represent riffs on the indoleethylamine core of 5-HT. For example, compound A (Table 11) employs N,N-dimethyltryptamine as its core, resulting in a molecule that has slightly lower affinity for the 5-HT_{1F} receptor compared with LY334370 but overall greater selectivity. Compounds B, C, D, and E illustrate that the indole nucleus can be replaced with other bicycles (e.g., azaindole, indazole, and indoline), resulting in compounds with very good 5-HT_{1F} receptor affinity and selectivity.

2. Partial Agonists and Antagonists. There are no published SAR studies for 5-HT_{1F} receptor antagonists. Methiothepin has been used in in vitro functional studies, but it is nonselective and of only moderate affinity for the 5-HT_{1F} receptor (Adham et al., 1993b). 1-naphthylpiperazine and metergoline were described as partial agonists to inhibit adenylyl cyclase activity (Adham et al., 1993a). Other compounds have been reported as partial agonists at 5-HT_{1F} receptor–stimulated [^{35}S]GTPγS binding, such as dihydroergotamine (68% of 5-HT; Wainscott et al., 1998), LY302148 (17% of 5-HT; Wainscott et al., 1998), frovatriptan (46% of 5-HT; Nelson et al., 2010), 2-thienyl-LYX (61% of 5-HT; Filla et al., 2003), and 2-pyridyl-LYX (63% of 5-HT; Filla et al., 2003).

The prototypical triptan, sumatriptan, has relatively high affinity for the 5-HT_{1F} receptor, and for comparison with the selective 5-HT_{1F} compounds, Table 6 lists the affinities of a number of triptans across the 5-HT receptor subtypes. In efficacy studies using a [^{35}S]GTPγS binding system to measure functional activity at the 5-HT_1 family of receptors, naratriptan, rizatriptan, sumatriptan, and zolmitriptan were found to act as full agonists at all the 5-HT_1 receptors that they stimulated. Frovatriptan, the only carbazole (i.e., a three-ring system containing indole), was a full agonist at 5-HT_{1B} and 5-HT_{1D} receptors but only a partial agonist at 5-HT_{1A} and 5-HT_{1F} receptors (Nelson et al., 2010). Hence, several triptans have high affinity for the 5-HT_{1F} receptor and may activate 5-HT_{1F} receptors in vivo at doses sufficient to also activate 5-$HT_{1B/1D}$ receptors (as well as other receptors; Table 6).

E. Signal Transduction

The 5-HT_{1F} receptor inhibits forskolin-stimulated adenylyl cyclase activity in recombinant cell systems

TABLE 10
Affinities of selective 5-HT_{1F} receptor agonists at cloned human 5-HT receptors

Binding values are expressed as K_i in nanomolar (± S.E.M. where available) from studies using cloned human receptors. Values in parentheses are the number of individual determinations from the cited references.

	LY302148	LY306258	LY334370[a]	LY344864[b]	LY397584	Lasmiditan[a] (COL-144, LY573144)
Receptor	K_i, nM	K_i, nM	K_i ± S.E.M., nM	K_i, nM	K_i, nM	K_i ± S.E.M., nM
5-HT_{1A}	12.0[c]	5370[c]	16.4 ± 2.7 (9)	530	1046[d]	1053 ± 134 (8)
5-HT_{1B}	53.7[e]	1698[e]	189 ± 25 (10)	549	723[d]	1043 ± 124 (8)
5-HT_{1D}	21.9[e]	794.3[e]	281 ± 58 (10)	575	724[d]	1357 ± 156 (8)
5-ht_{1e}	50.1[c]	73.6[f]	176 ± 34 (8)	1415	776[c]	594 ± 59.1 (6)
5-HT_{1F}	**2.5**[e]	**10.2**[e]	**1.87 ± 0.34 (10)**	**6**	**5.5**[d]	**2.21 ± 0.22 (8)**
5-HT_{2A}	5.89[c]	1072[c]	1530 ± 200 (3)	3935	3890[c]	>5 μM (6)
5-HT_{2B}	6.17[c]	1072[c]	1280 ± 90 (3)	1695	6166[c]	>2 μM (6)
5-HT_{2C}	13.5[c]	813[c]	3250 ± 930 (3)	3499	309[c]	>3 μM (6)
5-HT_6			>3 μM (2)			>4 μM (6)
5-HT_7			1550 ± 260 (5)	4851		>3 μM (6)

[a]Taken from Nelson et al. (2010).
[b]Taken from Phebus et al. (1997).
[c]Taken from Johnson (2005).
[d]Taken from Ramadan et al. (2003) (structure not reported).
[e]Taken from Johnson et al. (1997).
[f]Taken from Flaugh et al. (1998).

TABLE 11
Affinities of selective 5-HT_{1F} receptor agonists from SAR studies at cloned human 5-HT receptors
Binding values are expressed as K_i in nanomolar from studies using cloned human receptors.

	Compound A[a]	Compound B[b]	Compound C[c]	Compound D[d]	Compound E[e]
Receptor	K_i ± S.E.M., nM	K_i ± S.E.M., nM	K_i ± S.E.M., nM	K_i, nM	K_i, nM
5-HT_{1A}	265 ± 99	620 ± 37	1000 ± 270	870	240
5-HT_{1B}	1060 ± 204	270 ± 47	720 ± 220	2300	>10 μM
5-HT_{1D}	1620 ± 100	250 ± 38	720 ± 55	3100	2300
5-ht_{1e}	>4830	660 ± 110	770 ± 80		
5-HT_{1F}	**8.2 ± 1.2**	**5.0 ± 0.5**	**5.5 ± 0.6**	**3.9**	**9**
5-HT_{2A}	1000 ± 85	4800 ± 2000	3400 ± 1600		
5-HT_{2B}	676 ± 118	>10,000	>10,000		
5-HT_{2C}	2200 ± 390	1900 ± 1600	310 ± 41		
5-HT_4		4000 ± 350	2400 ± 740		
5-HT_6	380 ± 40	>10,000	1120 ± 203		
5-HT_7	1770 ± 130	>5000	Not determined		

[a]N-[3-(2-(dimethylamino)-ethyl)-2-methyl-1H-indol-5-yl]-4-fluorobenzamide, from Xu et al. (2001).
[b]N-(3-(1-methyl-4-piperidinyl)-1H-pyrrolo(3,2-b)pyridin-5-yl)acetamide, from Filla et al. (2003).
[c]N-[3-(1-Methyl-4-piperidinyl)-1H-pyrrolo[3,2-b]pyridin-5-yl]propanamide, from Filla et al. (2003).
[d]4-Fluoro-N-[3-(1-methyl-4-piperidinyl)-1H-indazol-5-yl]benzamide, from Zhang et al. (2004).
[e]4-Fluoro-N-[3-(1-methyl-4-piperidinyl)-2,3-dihydro-1H-indol-5-yl]benzamide, from Zhang et al. (2004).

(Amlaiky et al., 1992; Adham et al., 1993b), with later studies suggesting coupling through Gi/Go proteins based on pertussis toxin sensitivity (Adham et al., 1993a). The human 5-HT_{1F} receptor can also mediate inositol phosphate production and calcium flux through a pertussis toxin–sensitive mechanism in a recombinant system (Adham et al., 1993a). There are no reports on the transduction mechanisms affected by the 5-HT_{1F} receptor in native tissues.

F. Function

As noted above, signal transduction studies with heterologously expressed 5-HT_{1F} receptor readily identify responses, such as inhibition of forskolin-stimulated adenylyl cyclase activity (Amlaiky et al., 1992; Adham et al., 1993b), stimulation of inositol phosphate production and cellular calcium flux (Adham et al., 1993a), and stimulation of [^{35}S]GTPγS binding (Wainscott et al., 1998).

Neurogenic dural inflammation has been used as a model for the development of antimigraine drugs, in which the triptans display efficacy. Early dogma considered the mechanism of action of the triptans to be exclusively through the activation of 5-$HT_{1B/1D}$ receptors (Buzzi and Moskowitz., 1991; Humphrey, 2007). Yet other studies (Johnson et al., 1997; Wainscott et al., 1998) demonstrate that the pharmacologic profile for the inhibition of neurogenic dural inflammation in the guinea pig correlated better with the pharmacology of the 5-HT_{1F} receptor. Several additional studies support that 5-HT_{1F} receptor–selective agonists inhibit neurogenic dural inflammation; LY344864 and lasmiditan inhibit neurogenic dural inflammation in the rat (Phebus et al., 1997; Nelson et al., 2010), and "Compound A, B, and C" likewise in the guinea pig (Xu et al., 2001; Filla et al., 2003).

Several studies have examined the effects of 5-HT_{1F} receptor agonists on *c-fos* levels in the trigeminal system. Intracisternal administration of capsaicin in rats stimulates *c-fos* production in the trigeminal nucleus caudalis, an effect that is inhibited by pretreatment with either sumatriptan or LY344864 (Mitsikostas et al., 1999). The effect of sumatriptan, but importantly not that of LY344864, was blocked by a rat 5-HT_{1B} receptor antagonist. The inhibition of capsaicin-induced *c-fos* production by LY344864 and sumatriptan was also reproduced in the mouse (Mitsikostas et al., 2002). Electrical stimulation of the trigeminal ganglion in the rat induces *c-fos* production in the nucleus caudalis, which was blocked by lasmiditan in a dose-dependent fashion (Nelson et al., 2010). LY334370 was also active in this model.

Other models of neuronal stimulation have shown that 5-HT_{1F} receptor agonists produce inhibitory effects. For example, LY344864 inhibited a CGRP-mediated vasodepressor response produced by electrical stimulation of primary sensory nerves in the rat (González-Hernández et al., 2011). It did not affect vasodepressor responses induced by exogenously administered CGRP, consistent with the concept that prejunctional 5-HT_{1F} receptors inhibit CGRP release. LY344864 inhibited potassium-stimulated release of CGRP in the dura mater of the rat but not in the trigeminal ganglion or trigeminal nucleus caudalis (Amrutkar et al., 2012). Additionally, LY344864 inhibited activation of second-order neurons in the trigeminal nucleus caudalis elicited by electrical stimulation of the dura mater in rats (Shepheard et al., 1999).

Little work has been devoted to potential peripheral actions of 5-HT_{1F} receptors. Granados-Soto et al. (2010) have suggested that 5-HT_{1F} receptors are involved in peripheral pain mechanisms, as LY344864 blocked nociception induced by formalin injection into the rat paw. In a more direct examination of peripheral tissues LY334370 and LY344864 stimulated the production of markers of mitochondrial biogenesis in isolated rabbit renal proximal tubules, which was diminished when the tubule preparation was subject to 5-HT_{1F} receptor knockdown by siRNA transfection (Garrett et al., 2014). Administration of LY344864 in vivo to mice also led to an increase in a panel of markers for mitochondrial biogenesis in renal cortex, heart, and liver (Garrett et al., 2014).

Extensive study has failed to demonstrate that 5-HT_{1F} receptors contract blood vessels (e.g., Johnson et al., 1997; Cohen and Schenck, 1999, 2000; Razzaque et al., 1999; Shepheard et al., 1999; Bouchelet et al., 2000; Nelson et al., 2010). As detailed in *III. 5-HT_{1B} Receptors*, this contrasts functions associated with the 5-HT_{1B} receptor, through which triptans act to contract certain vascular tissues, including coronary artery, that can present serious adverse effects for patients, hence the optimism for 5-HT_{1F} receptor agonists as treatments for migraine with a reduced side-effect profile (see below).

G. Clinical Relevance and Therapeutics

As discussed above, because 5-HT_{1F} receptor agonism correlates with the pharmacology of the inhibition of a model of neurogenic dural inflammation—combined with the apparent absence of the 5-HT_{1F} receptor to contract vasculature—much of the translatable work concerning the 5-HT_{1F} receptor has centered on the treatment of migraine. This led, ultimately, to the development of LY334370, the first selective 5-HT_{1F} receptor agonist examined clinically for which efficacy to treat the pain of acute migraine attacks was noted (Goldstein et al., 2001). However, development of LY334370 was terminated because of safety concerns identified in animal toxicology studies (Ramadan et al., 2003; Ramadan and Buchanan, 2006). Further efforts to search for a more selective 5-HT_{1F} receptor agonist with preclincal toxicology issues identified LY573144. This molecule was subsequently out-licensed to CoLucid Pharmaceuticals (becoming COL-144; lasmiditan; Table 10), although the acquisition of CoLucid in 2017 by Eli Lilly returned the molecule to the parent company.

Lasmitidan is efficacious in alleviating symptoms of acute migraine in clinical trials. The first peer-reviewed published trial was a proof-of-concept investigation that demonstrated the efficacy of lasmiditan given intravenously (Ferrari et al., 2010). The primary efficacy measure was headache relief 2 hours after administration; a significant dose-response effect on efficacy separated lasmitidan from placebo. A trial using oral lasmiditan used the same primary efficacy measure, and all doses significantly improved headache response at 2 hours compared with placebo (Färkkilä et al., 2012). The most common side effects were dizziness, fatigue, vertigo, and paresthesia. Positive results from two pivotal phase III trials of lasmitidan (Kuca et al., 2018; Loo et al., 2019) led to subsequent marketing approval in 2019.

VII. 5-HT_{2A} Receptors

A. Introduction

The 5-HT_{2A} receptor (formerly 5-HT_2) was first identified as a binding site in rat brain with high (nanomolar) affinity for [^{3}H]spiperone and [^{3}H]ketanserin and low (micromolar) affinity for 5-HT (Peroutka and Snyder, 1979; Leysen et al., 1981). Soon after its discovery, the 5-HT_{2A} receptor was found to mediate several effects of 5-HT in the periphery, including platelet aggregation (De Clerck et al., 1982) and smooth muscle contraction (Cohen et al., 1981; Maayani et al., 1984; Engel et al., 1985). The peripheral 5-HT_{2A} receptors were originally classified as "D-type" 5-HT receptors based on pharmacological evidence (Bradley et al., 1986). The 5-HT_{2A} receptor was also the first 5-HT receptor found to couple to stimulate phosphatidyl inositol hydrolysis (Conn and Sanders-Bush, 1984).

B. Cloning of the Gene

The first 5-HT_{2A} receptor clone was isolated from rat brain cDNA libraries by homology screening based on the sequence of structurally related 5-HT_{2C} receptor (Pritchett et al., 1988; Julius et al., 1990). Functional expression of the cloned receptor confirmed coupling to phosphoinositide hydrolysis and Ca^{2+} mobilization. The human 5-HT_{2A} receptor was subsequently cloned by Saltzman et al. (1991) and displayed 87% homology with the rat receptor. The receptor contains 471 amino acids, with five potential glycosylation sites in the N-terminal extracellular domain and 11 potential phosphorylation sites in the C-terminal intracellular domain. The *HTR2A* gene encoding the human 5-HT_{2A} receptor has been mapped to chromosome 13q14–q21 (Sparkes et al., 1991). Analysis of the genomic structure of the human 5-HT_{2A} receptor revealed that it contains three exons separated by two introns, spanning more than 20 kb (Chen et al., 1992; Stam et al., 1992). Other species from which the 5-HT_{2A} receptor has been cloned include hamster (Van Obberghen-Schilling et al., 1991), mouse (Yang et al., 1992), and pig and rhesus monkey (Johnson et al., 1995) (Table 12). Sequence alignments for the 5-HT_{2A} receptor from eight species are shown in Fig. 8.

1. Regulation of 5-HT_{2A} Receptor Gene Expression. The structure of the 5-HT_{2A} promoter region has been characterized in humans, rats, and mice; the promoters lack canonical TATA or CAAT boxes. Fragments of a 1.6-kb segment from the 5′ flanking region of the human gene showed promoter activity when transfected into receptor-expressing human cell lines (Zhu et al., 1995). The human promoter sequence contains multiple transcription initiation sites, along with several binding sites for transcription factors, including simian virus 40 promoter factor 1, polyomavirus enhancer activator 3, cAMP response element, and E-box binding proteins. There was also evidence that the 5′ flanking sequence contains an alternative promoter as well as a silencing element upstream from the translation start codon. Falkenberg et al. (2011) subsequently demonstrated that the human promoter contains a glucocorticoid receptor (GR) binding site at position −1420. Furthermore, the A-allele of the −1438G/A (rs6311) polymorphism is believed to create a binding site for the

TABLE 12
5-HT_{2A} receptor genes, transcripts, and proteins

Organism	Gene		mRNA Transcript		Receptor Protein	
	Location	Ensembl Gene ID	NCBI RefSeq ID	Base Pairs	NCBI RefSeq ID	Amino Acids (aa)
Bos taurus (cow)	Ch 12: 16.82–16.88 Mb	ENSBTAG00000013498	NM_001001157	3562 bp	NP_001001157	470 aa
Equus caballus (horse)	Ch 17: 23.89–23.95 Mb	ENSECAG00000024282	NM_001081784	1413 bp	NP_001075253	470 aa
Homo sapiens (human)	Ch 13: 46.83–46.89 Mb (13q14–q21)	ENSG00000102468	NM_000621	5429 bp	NP_000612	471 aa
Macaca mulatta (rhesus macaque)	Ch 17: 25.98–26.05 Mb	ENSMMUG00000004210	NM_001032966	1438 bp	NP_001028138	471 aa
Mus musculus (mouse)	Ch 14: 74.64–74.70 Mb	ENSMUSG00000034997	NM_172812	2971 bp	NP_766400	471 aa
Rattus norvegicus (rat)	Ch 15: 56.66–56.73 Mb (15q11)	ENSRNOG00000010063	NM_017254	1566 bp	NP_058950	471 aa
Sus scrofa (pig)	Ch 11: 20.89–20.95 Mb	ENSSSCG00000009406	NM_214217	1432 bp	NP_999382	470 aa

transcription factor Th1/E47, which reportedly increases promoter activity (Smith et al., 2008).

Multiple *cis* elements in the mouse 5-HT_{2A} promoter act in a dynamic manner to regulate transcription. The 5′ flanking region of the mouse 5-HT_{2A} receptor gene contains a basal promoter (located −0.6 to −2.3 kb from the translational start site), which includes 11 transcription initiation sites, and binding sites for AP-2 (activating protein 2), polyomavirus enhancer activator 3, and simian virus 40 promoter factor 1 transcription factors (Ding et al., 1993; Toth et al., 1994). The activity of the basal promoter is attenuated in nonneuronal cells by two upstream repressor elements (extending from −2.3 to −4.2 kb), domains that presumably contain binding sites for transcription-inhibiting factors present in nonneuronal but not in neuronal cell types. Repressed genes can be reactivated in particular cell types via cell-specific activators, which may be responsible for 5-HT_{2A} receptor expression in certain nonneuronal cells. For example, Ding et al. (1993) identified a domain located upstream (−4.2 to −5.6 kb) from the repressor elements that reactivates transcription of the 5-HT_{2A} gene in C6 glioma cells.

The organization of the rat 5-HT_{2A} promoter is similar to that of mice, containing multiple negative and positive regulatory elements. Garlow et al. (1994) identified a primary transcription initiation site −1173 from the translational start site and a minimal promoter sequence in the 0.2-kb sequence immediately upstream from the primary initiation site. The activity of the minimal promoter is enhanced by proximal positive transitional elements 0.2–1.1 kb from the initiation site and attenuated by two distal negative domains located further upstream (1.1–2.2 and 2.3–2.5 kb from the initiation site, respectively). Analysis of the promoter and enhancer sequences revealed the presence of binding sites for the transcription factors nuclear factor 1, AP-1, AP-2, and Egr 1 as well as a GR element (Garlow et al., 1994; Garlow and Ciaranello, 1995). Experiments with transfected promoter-reporter plasmids showed that dexamethasone and AP-1 affected transcription of the promoter (Garlow and Ciaranello, 1995). The GR element appears to regulate 5-HT_{2A} receptor transcription in rat brain, as evidenced by the significant increase in 5-HT_{2A} mRNA expression induced by GR knockdown (Islam et al., 2004). AP-1 may play a role in agonist-induced upregulation of the 5-HT_{2A} receptor in rat cerebellar granule cells (Chalecka-Franaszek et al., 1999). In contrast, Du et al. (1994, 1995) reported that the primary 5-HT_{2A} transcriptional start site in rat myometrial smooth muscle cells is located at position −1120 from the translational start site. They also identified a basal promoter and two upstream repressor domains, but no enhancer region was detected. These discrepant findings may reflect cell type–specific differences in the function of the rat 5-HT_{2A} promoter.

C. Distribution

Many cell types in peripheral tissues express 5-HT_{2A} receptors, including platelets, fibroblasts, lymphocytes, and myocytes. In the CNS, neurons are the main site of localization, although the presence of 5-HT_{2A} receptors on nonneuronal cells types (glia, astrocytes) has also been reported (see below). The localization of 5-HT_{2A} receptors in the brain has been mapped by a combination of receptor autoradiography, in situ hybridization, immunocytochemistry, and, more recently, PET neuroimaging. Receptor autoradiography studies using [^{3}H]spiperone, [^{3}H]ketanserin, [^{125}I]DOI, and [^{3}H]MDL 100907 as radioligands have revealed high levels of 5-HT_{2A} receptor binding sites in many forebrain regions, including cortical and hippocampal areas, the basal ganglia, and olfactory tubercle, and the pattern is similar across species (e.g., Pazos et al., 1987b and López-Giménez et al., 1997). The distribution of 5-HT_{2A} receptor binding sites agrees well with that of 5-HT_{2A} mRNA (Mengod et al., 1990b; Morilak et al., 1994; Burnet et al., 1995), suggesting that 5-HT_{2A} receptors are largely expressed in the region of the somatodendritic and not trafficked along axons; however, there are some conflicting immunohistochemical data (for review, see Weber and Andrade, 2010; Nocjar et al., 2015). Moreover, much 5-HT_{2A} receptor immunoreactivity in rat neocortex has been detected in the cytoplasmic rather than membrane-bound compartments (Cornea-Hébert et al., 1999, 2002), which might reflect a high

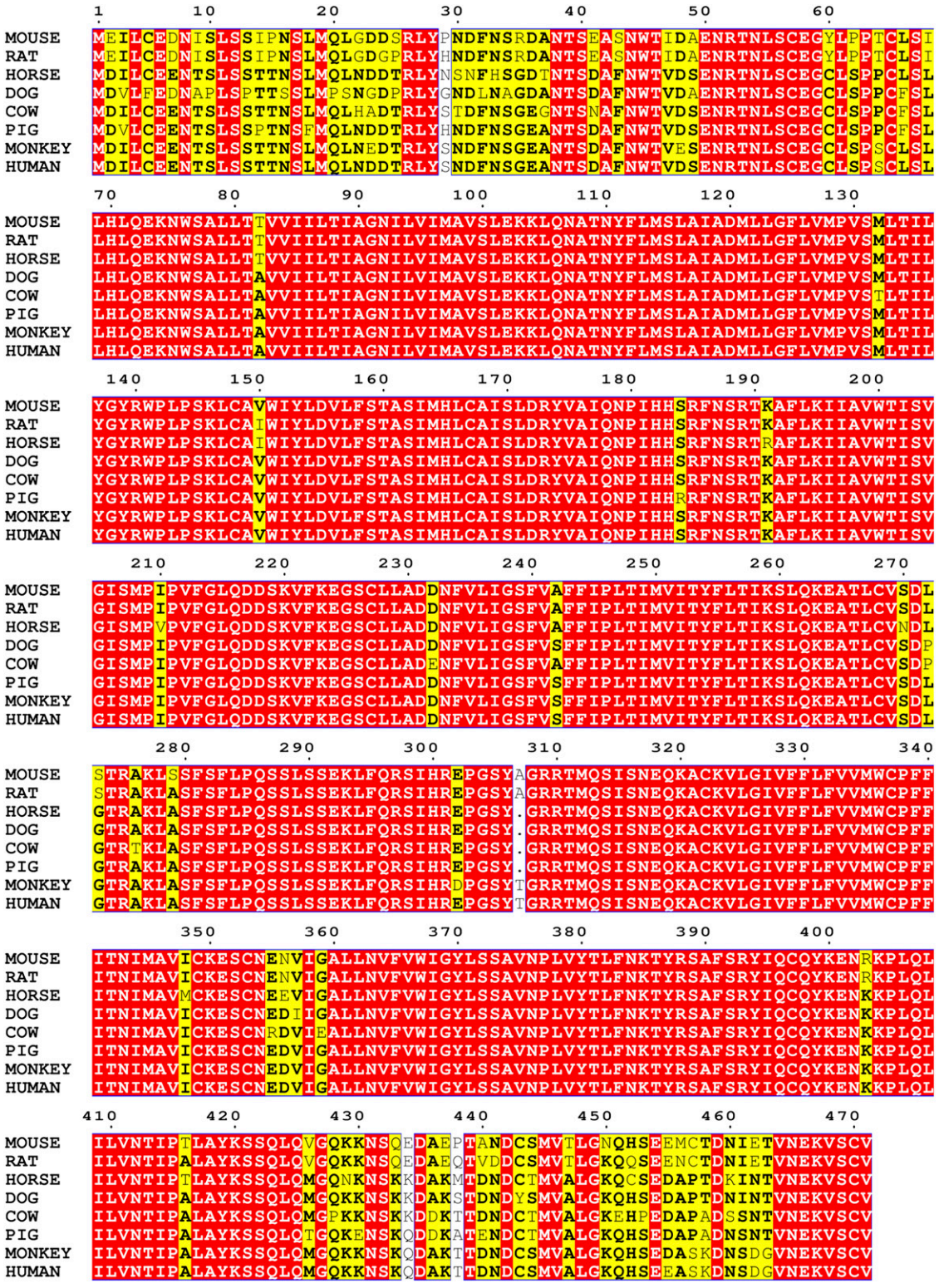

Fig. 8. Primary structure of 5-HT_{2A} receptors from various species.

intracellular reserve of the 5-HT_{2A} receptors and could be useful for the dynamic insertion of these receptors into the membrane.

A combination of immunocytochemical and in situ hybridization studies have investigated the cell types expressing the 5-HT_{2A} receptor in cerebral cortex (Fig. 9). Early data demonstrated the presence of 5-HT_{2A} receptors in cortical glutamatergic pyramidal (projection) neurons (Burnet et al., 1995), which have subsequently been mapped to specific cortical pathways (Vázquez-Borsetti et al., 2009; Mocci et al., 2014). Most such studies indicate that these cortical 5-HT_{2A}

receptors are predominantly postsynaptic and localized to either the apical dendrites or soma of pyramidal neurons. However, 5-HT_{2A} receptors have also been detected in GABAergic interneurons in the cortex (Morilak et al., 1994; Burnet et al., 1995; Mengod et al., 2015) and amygdala. There has also been an immunohistochemical analysis of 5-HT_{2A} receptor localization in the ventral tegmental area, and the majority of immunolabeling was colocalized with tyrosine hydroxlyase, suggesting that the receptors are expressed on dopaminergic neurons; however, there is also evidence for localization on VTA GABA neurons (Doherty and Pickel, 2000; Nocjar et al., 2002).

More recently, 5-HT_{2A} receptor localization has been mapped using bacterial artificial chromosome (BAC) transgenic mice engineered to express a fluorescent reporter (enhanced green fluorescent protein) under the control of the 5-HT_{2A} receptor promoter, thus revealing 5-HT_{2A} expression (Weber and Andrade, 2010). These data show a striking pattern of 5-HT_{2A} receptor distribution at the regional and cellular levels. Mapping within the cortical microcircuitry revealed 5-HT_{2A} receptor expression in specific lamina and in both pyramidal and interneurons. Interestingly, and in agreement with previous observations (Puig et al., 2010), expression was marked in cortical parvalbumin-positive interneurons, which underpin the formation of certain network oscillations (gamma frequency) thought critical for sensory information processing. The BAC transgenic mouse study and previous immunocytochemical studies (Stein et al., 2000; Weber and Andrade, 2010) also found 5-HT_{2A} receptors are located on parvalbumin-containing interneurons in the basolateral nucleus of the amygdala. This finding is consistent with data from electrophysiological studies showing that in the amygdala, 5-HT acts on 5-HT_{2A} receptors to potentiate GABAergic inhibition, including the GABA input to pyramidal neurons in this region (Jiang et al., 2009; Bocchio et al., 2015).

The study of BAC transgenic mice with enhanced green fluorescent protein under the control of the 5-HT_{2A} receptor promoter (Weber and Andrade, 2010) did not report the presence of 5-HT_{2A} receptors in nonneuronal cells, as suggested in earlier immunocytochemical studies (Xu and Pandey, 2000); however, further confirmation is awaited. Colocalization of 5-HT_{2A} receptors with other 5-HT receptor subtypes has been reported (5-HT_{1A}, 5-HT_{2C}; e.g., Puig et al., 2010; Stephens et al., 2014; Mengod et al., 2015; Nocjar et al., 2015; Tian et al., 2016), providing further evidence of potential crosstalk in 5-HT signaling at the receptor level.

The development of a number of 5-HT_{2A} receptor–selective radioligands has been useful for research tools, such as the imaging of 5-HT_{2A} receptors in humans, with the most successful including the single-photon emission computerized tomography radioligand [^{123}I] R91150 and the PET radioligands [^{18}F]setoperone, [^{18}F]-altanserin, and [^{11}C]MDL 100907 (Paterson et al., 2013; Herth and Knudsen, 2015). The first 5-HT_{2A} receptor agonist PET ligand, [^{11}C]*N*-(2-methoxybenzyl)-2,5-dimethoxy-4-bromophenethylamine ([^{11}C]Cimbi-36), has recently been reported (Ettrup et al., 2014) and raised

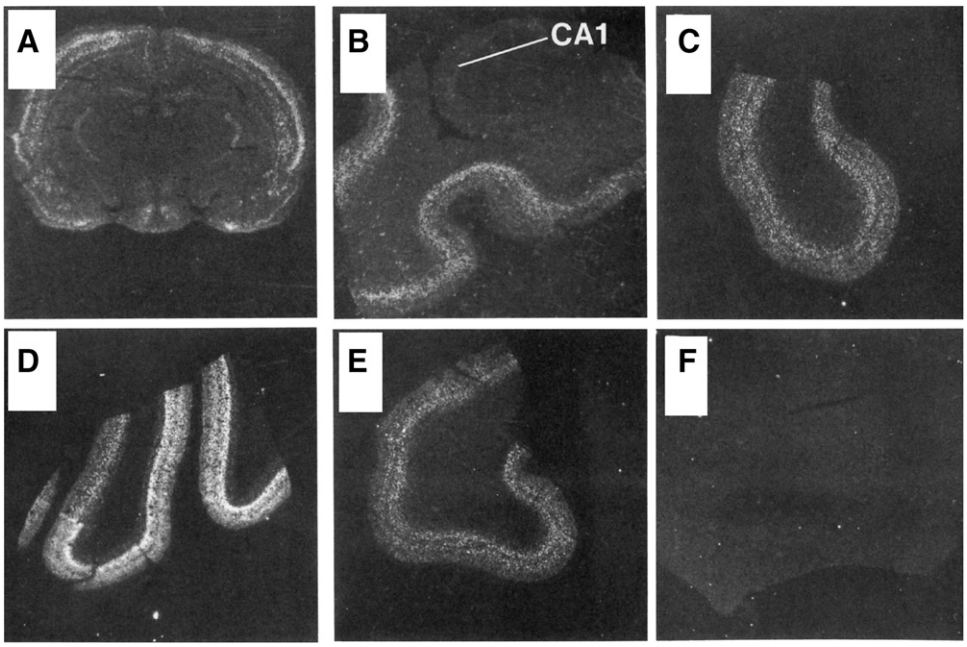

Fig. 9. In situ hybridization detection of 5-HT_{2A} receptor mRNA expression in rat and human brain. Reverse autoradiograms of the rat (A) and human brain (B–F). Human section: hippocampus and surrounding cortex (B), orbitofrontal cortex (Brodmann area 11) (C), striate cortex (Brodmann area 17) (D), superior temporal gyrus (Brodmann area 22) (E), and brainstem at the level of the raphe nucleus (F); no lack of 5-HT_{2A} receptor mRNA was evident. Adapted from Burnet et al. (1995) (with permission).

the interesting possibility that this may be displaceable by endogenous 5-HT and therefore provide an index of 5-HT release. [^{18}F]Altanserin PET has also been used to quantify 5-HT release (Quednow et al., 2012). A potential confound for the development of 5-HT$_{2A}$ receptor PET ligands is the reported high levels of 5-HT$_{2A}$ receptors in the intracellular compartment (see above). If the significant levels of PET binding are intracellular, then it is less likely to be in a position to be displaced by endogenous 5-HT. However, collectively, these imaging studies confirm the cross-species localization of 5-HT$_{2A}$ receptor and, more importantly, have opened the way for investigations of 5-HT$_{2A}$ receptors in disease states.

D. Post-translational Modifications and Impact

N-Glycosylation is known to regulate the intracellular sorting, surface expression, ligand binding, and signal transduction of GPCRs (Couvineau et al., 1996; Michineau et al., 2004). The extracellular *N*-terminus of the 5-HT$_{2A}$ receptor contains five potential *N*-glycosylation sites; glycosylation is apparently required for the 5-HT$_{2A}$ receptor to be targeted to the cell surface (Maginnis et al., 2010). Multiple proteins have been shown to interact with the 5-HT$_{2A}$ receptor (Table 13; see also *XVII. 5-HT GPCRs and their Interacting Proteins*).

E. Pharmacology

The three members of the 5-HT$_2$ receptor family share significant sequence homology (Fig. 10). Depending on the species examined, the seven transmembrane domains of 5-HT$_{2A}$ and 5-HT$_{2C}$ receptors display 79%–80% amino acid sequence conservation. Because of the high degree of structural homology, not surprisingly, 5-HT$_{2A}$ and 5-HT$_{2C}$ receptor binding affinities are highly correlated (Glennon et al., 1992a,b, 1994; Nelson et al., 1999). It is now recognized that most of the antagonists that have traditionally been used to block 5-HT$_{2A}$ receptors, including *N*-alkylpiperidines (e.g., ketanserin, ritanserin, pirenperone, and altanserin), ergolines (e.g., methysergide, metergoline, and LY53857), and tricyclic benzocycloheptenes (e.g., cyproheptadine and pizotifen), are also active at 5-HT$_{2C}$ receptor (Newton et al., 1996; Hoyer, 1988a,b). For example, altanserin is only 20-fold selective for 5-HT$_{2A}$ versus 5-HT$_{2C}$ receptor sites (Table 14). Ketanserin has been used extensively for reported pharmacological definition of 5-HT$_{2A}$ receptor responses and does show some selectivity for 5-HT$_{2A}$ receptor (pK_i = 8.7) compared with 5-HT$_{2B}$ (pK_i = 6.4) and 5-HT$_{2C}$ (pK_i = 6.8) receptors (Wainscott et al., 1996). However, ketanserin also has moderate affinity for adrenergic (α_1) and histaminergic (H$_1$) receptors as well as 5-HT$_{1D}$ receptors and the vesicular monoamine transporter (Erickson et al., 1996; Leysen et al., 1996; Bucholtz et al., 1999; Yoshio et al., 2001), which can complicate interpretation of arising data. Ritanserin is even less selective for 5-HT$_{2A}$ versus 5-HT$_{2C}$ receptors and also interacts with 5-HT$_{1D}$, 5-HT$_6$, 5-HT$_7$, D$_2$, D$_3$, D$_4$, H$_1$, and α_1 sites (Bard et al., 1993; Monsma et al., 1993; Shen et al., 1993; Leysen et al., 1996; Seeman and Tallerico, 1998; Yoshio et al., 2001). The butyrophenone neuroleptic spiperone displays 500- to 2000-fold selectivity for 5-HT$_{2A}$ versus 5-HT$_{2C}$ and has often been used to discriminate those receptors, but it binds to numerous other receptors, including dopaminergic D$_2$, D$_3$, and D$_4$; adrenergic α_1 and α_2; and 5-HT$_{1A}$ and 5-HT$_7$ receptors (Ruat et al., 1993b; Tang et al., 1994; Metwally et al., 1998; Corradetti et al., 2005). The spiperone derivative AMI-193 (8-[3-(4-fluorophenoxy)-propyl]-1-phenyl-1,3,8-triazaspiro[4,5]decan-4-one) is twice as selective as spiperone for 5-HT$_{2A}$ versus 5-HT$_{2C}$ but retains nanomolar affinity for D$_2$ and 5-HT$_{1A}$ (Ismaiel et al., 1993). Atypical antipsychotics such as risperidone, olanzapine, and clozapine block 5-HT$_{2A}$ receptors with high affinity but generally have limited selectivity versus 5-HT$_{2C}$ and dopamine receptors. By contrast, haloperidol and other typical antipsychotics have higher affinity for dopamine D$_2$ receptors than for 5-HT$_{2A}$ receptors.

The 4-carbinolpiperidines volinaserin (MDL 100907, M100907) and glemaserin (MDL 11939) were the first truly selective 5-HT$_{2A}$ receptor antagonists. Volinaserin

TABLE 13
Proteins reported to interact with the 5-HT$_{2A}$ receptor

Interacting Protein	Region of the 5-HT$_{2A}$ Receptor	Reference
ADP-ribosylation factor 1 (Arf1)	C-terminus (NPxxY motif)	Robertson et al., 2003; Johnson et al., 2006
β-arrestin	ICL3, C-terminus (ASK motif[a])	Gelber et al., 1999; Bhattacharya et al., 2010
Calmodulin (CaM)	ICL2, C-terminus	Turner and Raymond, 2005
Caveolin-1 (Cav-1)	ND	Bhatnagar et al., 2004
Glutamine synthetase	ICL3	Sheffler et al., 2006
Jak2 kinase	ND	Guillet-Deniau et al., 1997
Microtubule-associated protein 1A (MAP1A)	ICL3	Sheffler et al., 2006
Multi-PDZ domain protein 1 (MUPP1)	C-terminus (PDZ domain)	Jones et al., 2009
Na^+/H^+ exchange regulatory factor 3 (NHERF3)	ND	Walther et al., 2015
Nucleoside-diphosphate kinase 3 (NME3)	ICL3	Sheffler et al., 2006
Paraoxonase 2 (PON2)	ICL3	Sheffler et al., 2006
Postsynaptic density protein 95 kDa (PSD-95)	C-terminus (PDZ domain)	Xia et al., 2003
Protein phosphatase 5 (PP-5)	ICL3	Sheffler et al., 2006
Ribosomal S6 kinase 2 (RSK2)	ICL3	Sheffler et al., 2006
Synapse-associated protein 97 (SAP97)	C-terminus (PDZ domain)	Dunn et al., 2014

C-terminus, carboxyl-terminus; ICL2, second intracellular loop; ICL3, third intracellular loop; ND, not determined.
[a]Specific to the human and monkey 5-HT$_{2A}$ receptor.

has subnanomolar affinity for 5-HT_{2A} and 50- to 100-fold lower affinity for 5-HT_{2C} and α_1 receptors, with negligible affinity for other investigated sites (Palfreyman et al., 1993; Kehne et al., 1996). In contrast to most 5-HT_{2A} receptor antagonists, the selectivity of M100907 for 5-HT_{2A} versus 5-HT_{2C} receptors has been verified in mice (Canal et al., 2013). Compared with volinaserin, glemaserin displays even greater selectivity for 5-HT_{2A} receptor (K_i = 2.893 nM) versus 5-HT_{2B} (K_i = 1419 nM), 5-HT_{2C} (K_i = 853.6 nM), and α_1 (K_i = 588 nM) receptor sites (Dudley et al., 1988; Pehek et al., 2006). Like most drugs that block 5-HT_{2A} receptor responses, volinaserin and glemaserin were initially thought to be neutral antagonists but are now known to act as inverse agonists (Weiner et al., 2001; Aloyo et al., 2009). Although it was previously difficult to conclusively discriminate responses mediated by individual 5-HT_2 receptor subtypes in vitro and in vivo, volinaserin or glemaserin at appropriate concentrations in combination with selective 5-HT_{2B} and 5-HT_{2C} receptor antagonists, such as RS-127445 and SB-242084, respectively, allow such pharmacological investigations.

In contrast to initial reports, it is now recognized that the 5-HT_{2A} receptor has high affinity for 5-HT. For example, 5-HT competes for the agonist radioligand [^{3}H]DOB with a K_i of 6.13 nM (Titeler et al., 1985), and [^{3}H]5-HT reportedly radiolabels the 5-HT_{2A} receptor with K_d = 1.3 nM (Sleight et al., 1996). In contrast, competition binding experiments with antagonist radioligands tend to underestimate the affinity of 5-HT_{2A} receptor agonists; 5-HT_{2A} receptors exist in low-affinity and high-affinity agonist binding conformations depending on whether they are coupled to G proteins, and only a small fraction of 5-HT_{2A} receptors are in the G protein–coupled, agonist high-affinity conformation at any given time. 5-HT_{2A} receptor antagonists bind to both conformations with equal affinity (Lyon et al., 1987; Glennon et al., 1988). Therefore, the apparent affinity of 5-HT_{2A} receptor agonists varies depending on the intrinsic activity of the radioligand used to label the receptor, with agonists displaying 10- to 100-fold higher affinity for agonist-labeled receptors versus antagonist-labeled receptors

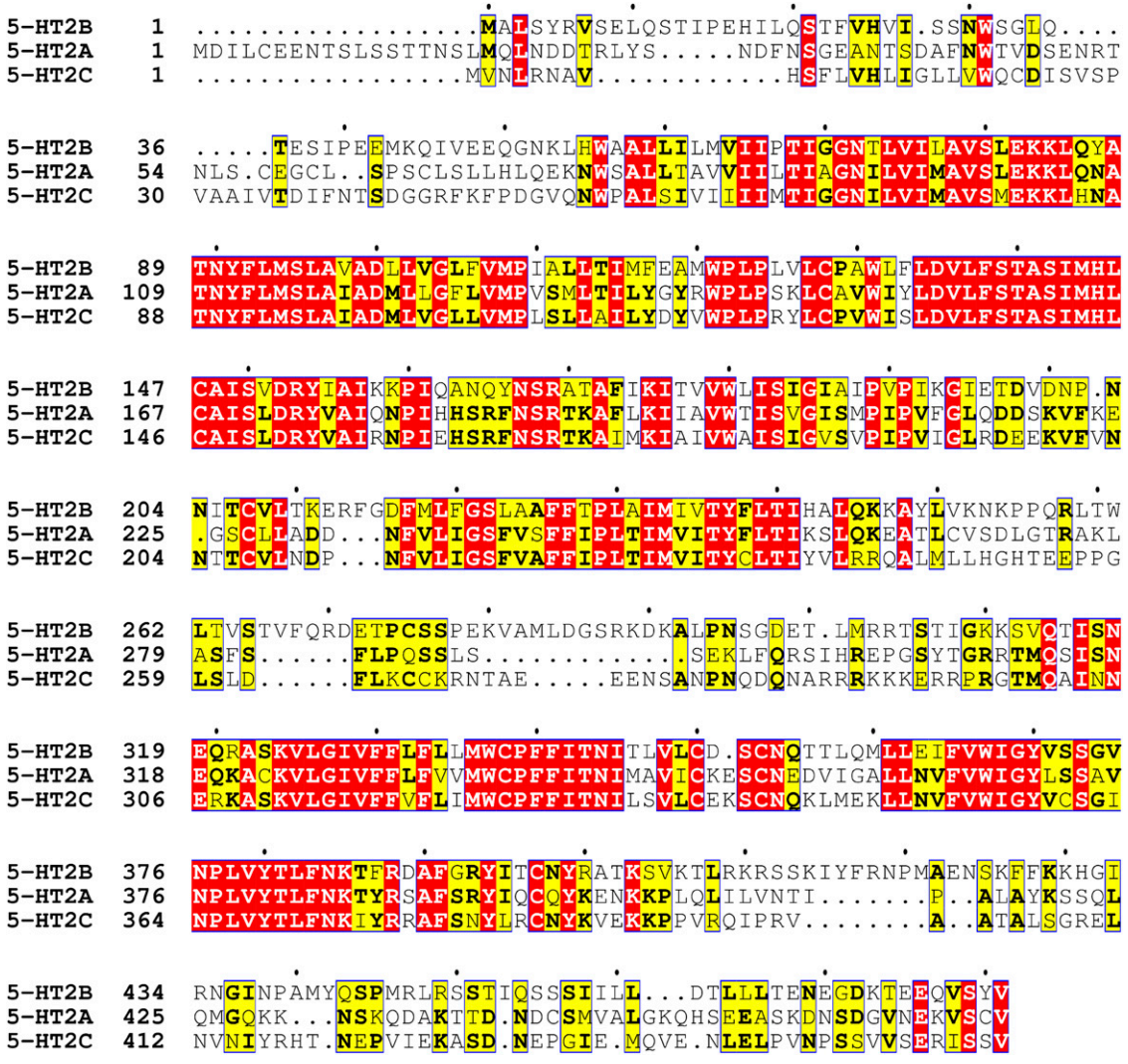

Fig. 10. Primary structure of human 5-HT_{2A}, 5-HT_{2B}, and 5-HT_{2C} receptors.

TABLE 14
Affinities of 5-HT_{2A} receptor antagonists

Ligand	h5-HT_{2A} K_i (nM)	r5-HT_{2A} K_i (nM)	h5-HT_{2C} K_i (nM)	r5-HT_{2C} K_i (nM)	hα_{1A} K_i (nM)	hα_{1B} K_i (nM)	References
Spiperone	1.4	0.43	661	871	5.0	0.6	Bonhaus et al., 1997; Yoshio et al., 2001
Ketanserin	3.2	1.3	200	63	6.3	6.3	Yoshio et al., 2001; Bonhaus et al., 1995
Ritanserin	0.25	0.30	0.25	2.7	4.0	10	Bonhaus et al., 1997; Yoshio et al., 2001
Pirenpirone	1.08		77				Wainscott et al., 1996
Risperidone	1.1	0.16	12	32	4.0	10	Yoshio et al., 2001; Roth et al., 1992; Kongsamut et al., 1996; Schotte et al., 1996
Altanserin	0.51	0.3		6.0			Smith et al., 1998; Tan et al., 1999
MDL 11,939	6.06	2.893	1020	853.6	1876	1579	Wainscott et al., 1996; Pehek et al., 2006
M100,907	1.50	1.92		88	128	424.7	Pehek et al., 2006; Kehne et al., 1996

h, human; r, rat.

(Titeler et al., 1987; Glennon et al., 1994; Sleight et al., 1996).

Numerous 5-HT_{2A} receptor agonists are available (Table 15). Tryptamines such as α-methyl-5-HT and 5-methoxy-*N*,*N*-dimethyltryptamine are widely used as 5-HT_{2A} receptor agonists, but they tend to activate 5-HT receptors nonselectively and can inhibit the 5-HT transporter at micromolar concentrations (Ismaiel et al., 1990; Nagai et al., 2007; Blough et al., 2014). 2,5-Dimethoxy-4-iodoamphetamine (DOI) and its structural analogs 2,5-dimethoxy-4-methylphenyl)-2-aminopropane, DOB, and (4-Bromo-3,6-dimethoxybenzocyclobuten-1-yl)-methylamine have high affinity and selectivity for 5-HT_2 receptors but do not discriminate between the three 5-HT_2 receptor subtypes. In contrast to most phenylalkylamines, which are nonselective for 5-HT_{2A} versus 5-HT_{2C} receptors, 25CN-NBOH (N-(2-hydroxybenzyl)-2,5-dimethoxy-4-cyanophenethylamine) is reportedly 100-fold selective for 5-HT_{2A} (K_i = 1.3 nM) versus 5-HT_{2C} receptors (K_i = 132 nM) (Hansen et al., 2014). The conformationally restricted phenethylamine (+)-(2*S*,6*S*)-*trans*-2-(2,5-dimethoxy-4-bromobenzyl)-6-(2-methoxyphenyl)piperidine displays even greater selectivity for 5-HT_{2A} receptors, binding to 5-HT_{2C} receptors with 124-fold lower affinity (Juncosa et al., 2013). Racemic *trans*-DMBMPP is less selective but still shows 98-fold higher affinity for 5-HT_{2A} over 5-HT_{2C} receptors.

F. Function

1. Signaling. The Gα_q–PLCβ cascade is the canonical signaling pathway coupled to 5-HT_{2A} receptor activation (Conn and Sanders-Bush, 1984; Kendall and Nahorski, 1985), resulting in the hydrolysis of membrane phospholipids to inositol-1,4,5-triphosphate (IP_3) and diacylglycerol (DAG). In turn, IP_3 elevates the concentration of Ca^{2+} in the cytosol by releasing it from the endoplasmic reticulum (ER), whereas DAG activates protein kinase C (PKC) and facilitates its translocation from the cytosol to the membrane. Both PKC and Ca^{2+} are known to have widespread and far-reaching influences on intracellular signaling; PKC phosphorylates various target proteins, whereas Ca^{2+} is known to modulate the activity of enzymes (e.g., Ca^{2+}/calmodulin-dependent kinases) and ion channels.

In addition to the classic signaling mediated by the PLC-IP_3 cascade, the 5-HT_{2A} receptor can activate a variety of other effector mechanisms. For example, the 5-HT_{2A} receptor has been shown to stimulate phospholipase A_2 (PLA_2), resulting in increased production of the second messenger AA (Felder et al., 1990; Berg et al., 1996). The 5-HT_{2A} receptor also increases release of the endocannabinoid 2-arachidolylglycerol (Parrish and Nichols, 2006). The 5-HT_{2A} receptor can also couple to an MAPK such as ERK1/2 (Hershenson et al., 1995; Watts, 1996; Greene et al., 2000). Other signaling molecules that have been linked to 5-HT_{2A} activation in native tissues and in cell lines expressing the receptor, including Akt (Johnson-Farley et al., 2005), phospholipase D1 (PLD) (Barclay et al., 2011), JAK/STAT (Guillet-Deniau et al., 1997; Banes et al., 2005), NOS (Miller et al., 1997), cAMP response element binding (CREB) (Chalecka-Franaszek et al., 1999), calmodulin (Turner and Raymond, 2005), and glycogen synthase kinase-3β (Li et al., 2004).

Some of the aforementioned signaling pathways are coupled to the 5-HT_{2A} receptor in a Gα_q-independent manner. The ability of the 5-HT_{2A} receptor to stimulate PLD is mediated by the monomeric G protein ADP-ribosylation factor-1 (ARF1), which interacts directly with the receptor (Barclay et al., 2011). The coupling of the 5-HT_{2A} receptor to PLA_2 in NIH3T3 cells appears to

TABLE 15
Affinities of 5-HT_{2A} receptor agonists

Ligand[a]	h5-HT_{2A} K_i (nM) [^{3}H]ketanserin	h5-HT_{2A} K_i (nM) [^{3}H]mesulergine	Reference
(±)-DOI	3.2	19.1	Canal et al., 2013
Cimbi-5 (25I-NBOMe)	0.52	0.69	Nichols et al., 2015
Cimbi-36 (25B-NBOMe)	0.19	4.0	Juncosa et al., 2013
(+)-(2*S*,6*S*)-DMBMPP	2.5	310	Juncosa et al., 2013
25CN-NBOH (NBOH-2C-CN)	2.2	49.8	Halberstadt et al., 2016

[a]Alternative names are shown in parentheses.

be mediated by two independent signaling cascades. In the first case, activation of *Src* by $G\alpha_{i/o}$-associated $G\beta\gamma$ subunits results the formation of a ternary complex between SHC/GRB/SOS, which in turn activates the Ras–Raf–MEK1/2–ERK1/2 cascade. In the second, activation of RhoA by $G\alpha_{12/13}$ stimulates p38 MAPK (Kurrasch-Orbaugh et al., 2003a). Reports also indicate PLC and PKC are not involved in the ERK activation produced by 5-HT_{2A} receptor agonists in PC12 and vascular smooth muscle cells (Florian and Watts, 1998; Banes et al., 1999; Quinn et al., 2002). Nevertheless, in certain cell types, the coupling of ERK1/2 to 5-HT_{2A} receptors is dependent on $G\alpha_q$. Activation of the Ras–Raf–MEK–ERK1/2 cascade by 5-HT_{2A} receptors in tracheal smooth muscle cells and mesangial cells is downstream from $G\alpha_q$ and dependent on PKC (Hershenson et al., 1995; Watts, 1996; Greene et al., 2000). The coupling of the 5-HT_{2A} receptor to MAPK may actually be ligand-specific. In mouse embryonic fibroblasts transfected with the 5-HT_{2A} receptor, the stimulation of ERK1/2 by DOI is dependent on PLC, whereas the stimulation by 5-HT is PLC-independent and requires β-arrestin2 (Schmid et al., 2008).

The 5-HT_{2A} receptor was one of the first receptors shown to display functional selectivity. According to Berg et al. (1998a,b), 5-HT_{2A} receptors activate PLC and PLA_2 independently in CHO cells, and the relative efficacies of agonists differ depending on which response is measured. Subsequent studies confirmed the PLC-IP_3 and PLA_2-AA pathways coupled to 5-HT_{2A} receptor in NIH3T3 cells have different receptor reserves, indicating they are activated independently (Kurrasch-Orbaugh et al., 2003b). It has been proposed that 5-HT_{2A} receptor functional selectivity (biased signaling) may explain why certain 5-HT_{2A} receptor agonists produce hallucinogenic effects, whereas other agonists such as lisuride are nonhallucinogenic (González-Maeso et al., 2007). Specifically, although both hallucinogenic and nonhallucinogenic 5-HT_{2A} receptor agonists increase the expression of the immediate-early gene *c-fos* by activating $G\alpha_q$, only agonists with hallucinogenic effects increase the cortical expression of the immediate-early gene (e.g., *r-2*) by activating $G\alpha_{i/o}$ and *Src*. Another group has reported that the activation of the 5-HT_{2A} receptor by 5-HT and hallucinogens results in vastly different downstream signaling responses (Schmid and Bohn, 2010). The behavioral response to 5-HT in mice requires Akt phosphorylation and formation of a complex between Akt, *Src*, and β-arrestin2, whereas the response to hallucinogens is independent of Akt and β-arrestin2.

It is now apparent that many 5-HT_{2A} receptor antagonists act in a functionally selective manner. Like most GPCRs, the 5-HT_{2A} receptor is downregulated by exposure to agonists. Somewhat paradoxically, however, prolonged exposure to certain 5-HT_{2A} receptor antagonists also induces 5-HT_{2A} receptor downregulation (Leysen et al., 1986; Eison et al., 1989; Pranzatelli, 1991; Moreno et al., 2013). The anomalous downregulation of 5-HT_{2A} receptors induced by antagonists is not a consequence of altered gene transcription (Roth and Ciaranello, 1991) but is likely caused by redistribution of the receptor from the cell surface to intracellular compartments (Willins et al., 1998, 1999; Bhatnagar et al., 2001). Interestingly, although the 5-HT_{2A} receptor antagonists ketanserin, clozapine, and olanzapine reduce 5-HT_{2A} immunoreactivity and [^{3}H]ketanserin binding in mouse frontal cortex, M100907, MDL 11939, and altanserin do not alter receptor expression (Yadav et al., 2011). These findings indicate that certain 5-HT_{2A} receptor antagonists have agonist-like effects on the signaling pathways responsible for promoting receptor internalization. For example, although ketanserin and risperidone act as inverse agonists on the 5-HT_{2A}–PLC and 5-HT_{2A}–ERK pathways, they also act as 5-HT_{2A} receptor agonists by stimulating β-arrestin translocation (Clarke et al., 2013). Nevertheless, it appears that 5-HT_{2A} receptor antagonists can promote 5-HT_{2A} receptor internalization through multiple mechanisms because clozapine does not recruit β-arrestin (Schmid et al., 2014). Clozapine does act in a functionally selective manner—it has been shown to induce Akt phosphorylation in cortical neuronal cultures via the 5-HT_{2A} receptor (Schmid et al., 2014); but the mechanism by which clozapine promotes receptor downregulation remains to be determined.

Adding to the complexity of 5-HT_{2A} receptor signaling is the discovery that it can oligomerize with other GPCRs, potentially allowing the 5-HT_{2A} receptor to couple to an even wider range of effector mechanisms. Gonzalez-Maseo et al. (2008) have reported that 5-HT_{2A} and $mGlu_2$ receptors form heterocomplexes, providing a mechanism whereby 5-HT_{2A} receptors can modulate $G\alpha_{i/o}$ signaling. Heteromers between 5-HT_{2A} and D_2 receptors (Borroto-Escuela et al., 2010; Lukasiewicz et al., 2010) and 5-HT_{2A} and CB_1 receptors (Viñals et al., 2015) have also been detected in transfected cells, but the functional significance of these interactions and the extent to which they occur in native tissues is unclear.

2. Action in Cells, Tissues, and In Vivo. 5-HT_{2A} receptor activation produces a variety of physiologic effects in peripheral tissues and in the CNS. There is evidence for 5-HT_{2A} receptor involvement in the proliferation, differentiation, and contraction of vascular and extravascular smooth muscle (Maayani et al., 1984; Watts and Cohen, 1992; Fanburg and Lee, 1997; Shum et al., 2002; Itoh and Kajikuri, 2011) as well as increased contractility of cardiac muscle, where it is expressed in neonatal and reexpressed in failing and hypertrophic heart (Qvigstad et al., 2005c; Birkeland et al., 2007b; Brattelid et al., 2007a,b, 2012; Levy et al., 2008). Similarly, the 5-HT_{2A} receptor increases the proliferation and synthesis of extracellular matrix proteins by glomerular mesangial cells (Kasho et al., 1998; Göoz et al., 2006). 5-HT_{2A} receptor antagonists inhibit the platelet

aggregation and shape change induced by 5-HT, DOB, and DOI (de Clerck et al., 1982, 1984; Seggel et al., 1987). The neuroendocrine effects of DOI in rats, including increases in the release of adrenocorticotropic hormone, corticosterone, oxytocin, renin, and prolactin, appear to be mediated by 5-HT_{2A} (Calogero et al., 1990; van de Kar et al., 2001; Zhang et al., 2002). 5-HT_{2A} receptor activation produces sympathoexcitatory effects and increases arterial pressure through a combination of central and peripheral effects (Tadepalli et al., 1975; McCall et al., 1987; Alper, 1990; Dedeoğlu and Fisher, 1991; Chaouche-Teyara et al., 1994).

5-HT_{2A} receptor activation produces long-lasting increases in the excitability and firing rate of glutamatergic and GABAergic neurons, resulting in increased excitatory and inhibitory network activity (McCormick and Wang, 1991; Sheldon and Aghajanian, 1991; Cumming-Hood et al., 1993; Pessia et al., 1994; Marek and Aghajanian, 1996; Aghajanian and Marek, 1997; Shen and Andrade, 1998; Zhou and Hablitz, 1999; Lambe and Aghajanian, 2006, 2007; Béïque et al., 2007; Benekareddy et al., 2010; Avesar and Gulledge, 2012; but see also Carr et al., 2002; Tian et al., 2016). These electrophysiological effects are absent in Htr2A-knockout mice and are restored upon conditional cortical rescue of 5-HT_{2A} receptors (Weisstaub et al., 2006). Reductions in resting K^+-conductances are thought to contribute to many of the excitatory effects of 5-HT_{2A} receptor activation. For example, 5-HT_{2A} receptor activation induces membrane depolarization, reduces afterhyperpolarization, and evokes a slow afterdepolarization in layer V pyramidal neurons in the PFC, effects thought to be mediated by inhibition of the Ca^{2+}-activated K^+-current $I_{K(Ca)}$ and activation of the Ca^{2+}-dependent nonselective cation current I_{CAN} (Araneda and Andrade, 1991; Tanaka and North, 1993; Villalobos et al., 2005, 2011; Zhang and Arsenault, 2005). The ability of 5-HT_{2A} receptor activation to enhance motoneuron excitability is mediated by inhibition of a leak K^+-current [$I_{K(leak)}$] and enhancement of the hyperpolarizing-activated nonselective cation current (Garratt et al., 1993; Hsiao et al., 1997; Larkman and Kelly, 1998; Xu et al., 2009). TASK-1 and TASK-3 two-pore domain K^+-channels are believed to be responsible for $I_{K(leak)}$ in motoneurons (Talley et al., 2000; Larkman and Perkins, 2005). Likewise, the 5-HT_{2A} receptor–mediated depolarization produced by 5-HT in entorhinal cortex interneurons occurs as a consequence of TASK-3 channel inhibition (Deng and Lei, 2008). 5-HT_{2A} has been shown to depolarize nucleus accumbens medium spiny neurons by inhibiting an inwardly rectifying K^+-current (North and Uchimura, 1989). There is also evidence that 5-HT_{2A} receptor–induced arterial constriction is mediated by inhibition of Kv channels (Cogolludo et al., 2006; Sung et al., 2013). Other ion channels known to couple to the 5-HT_{2A} receptor include rapidly inactivating and persistent voltage-dependent Na^+ channels (Carr et al., 2002), CaV 1.2 L-type Ca^{2+} channels (Day et al., 2002), Kv1.2 K^+ channels (Lambe and Aghajanian, 2001), and voltage-independent Ca^{2+} channels (Hagberg et al., 1998).

G. Clinical Relevance

5-HT_{2A} receptors are clinically relevant to numerous CNS disorders, ranging from mood disorder and schizophrenia to drug dependence. The links between the 5-HT_{2A} receptor and the causes of such CNS disorders are not well understood. Early life stress has been shown trigger strong upregulation of the functional effects of 5-HT_{2A} receptors on electrophysiology, signaling, and gene expression in prefrontal cortex (Benekareddy et al., 2010). Emerging findings regarding 5-HT_{2A} gene polymorphisms and epigenetic regulation of 5-HT_{2A} receptor expression are also of great interest. The association between the 5-HT_{2A} receptor and successful treatment of certain CNS disorders is strong, and there is an ongoing active research interest in the therapeutic potential of 5-HT_{2A} receptor ligands. The majority of these studies have had a particular focus on schizophrenia and depression and the actions of antipsychotic and antidepressant drugs.

In schizophrenia, there are reports of reduced 5-HT_{2A} receptor binding in frontal cortex of postmortem brains, and there is supporting evidence from PET imaging studies for similar changes, as recently reviewed (Selvaraj et al., 2014). However, given that not all the PET studies used 5-HT_{2A} receptor–selective radiotracers and that there are inconsistencies in the postmortem studies, the extent to which impaired cortical 5-HT_{2A} receptor function contributes to the symptoms of schizophrenia is unclear.

The psychotomimetic effects of hallucinogens such as LSD and psilocybin are undoubtedly linked to activation of 5-HT_{2A} receptors (Halberstadt, 2015). Furthermore, commonly used second-generation antipsychotics such as clozapine, risperidone, and sertindole are potent 5-HT_{2A} receptor antagonists in addition to having affinity at other 5-HT receptor subtypes and receptors for other neurotransmitters (Meltzer, 2012). 5-HT_{2A} receptor blockade has been linked to the antipsychotic efficacy of second-generation antipsychotics as well as a reduced side effect profile (Meltzer and Massey, 2011). This provided support for the idea that selective 5-HT_{2A} receptor antagonists might be useful as a monotherapy for some psychoses and as antipsychotic augmenting agents, although schizophrenia trials with the selective 5-HT_{2A} receptor antagonist MDL 100907 were disappointing. Nevertheless, the subsequent discovery that 5-HT_{2A} receptor inverse agonists have antipsychotic actions in preclinical models (Weiner et al., 2001) suggests an alternative way to treat psychosis. This thinking advanced to the clinical development of pimavanserin (Vanover et al., 2006) for the treatment of psychosis in patients with Parkinson disease (Meltzer and Roth, 2013). In 2016, pimavanserin was approved by the U.S. FDA as a treatment for hallucinations and delusions associated with Parkinson disease. Although the degree

of inverse agonism required for an antipsychotic effect is uncertain, 5-HT_{2A} receptor inverse agonists are currently considered as a potential new generation of antipsychotic agents.

In postmortem studies of suicide victims, there is evidence of an upregulation in 5-HT_{2A} receptors in cortex [for review, see Meyer (2013)]. This finding parallels results from PET imaging studies that report increased 5-HT_{2A} receptor binding in patients with depression, particularly in association with severe pessimism (Meyer et al., 2003; Bhagwagar et al., 2006). The latter observations fit with findings in healthy subjects, in that individuals with high scores for pessimistic personality had higher cortical 5-HT_{2A} receptor binding (Frokjaer et al., 2008). It has been hypothesized that the increase in cortical 5-HT_{2A} receptor binding in depression is associated with severe pessimism and arises as an adaptive response to chronic 5-HT deficiency (Meyer, 2013).

The above findings in patients with depression link to evidence that certain antidepressants, and particularly tricyclics, have high affinity for 5-HT_{2A} receptors (e.g., Millan, 2006). Though tricyclic antidepressants have high affinity for multiple receptors, it can be argued that evidence of the superior therapeutic efficacy of tricyclic antidepressant drugs over other antidepressant drug classes, particularly in the treatment of severe melancholic depression, is linked in part to their ability to block 5-HT_{2A} receptors. Thus, 5-HT_{2A} receptor antagonists augment the effect of 5-HT uptake inhibitors in preclinical models (Marek et al., 2005; Boothman et al., 2006), and drugs with 5-HT_{2A} receptor antagonist properties are advocated as an add-on to antidepressant therapy in treatment-resistant cases (Marek et al., 2003); all are a possible link to evidence of an inhibitory 5-HT_{2A} receptor–mediated feedback on 5-HT neurons (Sharp et al., 2007).

Aside from schizophrenia and depression, 5-HT_{2A} receptors are relevant to the pathophysiology of many other CNS disorders. Currently, there is large and ongoing research effort to understand the importance of polymorphic variation in the 5-HT_{2A} receptor gene to a large variety of psychiatric disorders (e.g., Smith et al., 2013; Paquette and Marsit, 2014). Despite the many reports of associations between 5-HT_{2A} gene polymorphisms and neuropsychiatric disorders, some of which are subject to careful meta-analysis, effect sizes are at best modest, and the role of genetic 5-HT_{2A} variability in mental health continues to be uncertain. This work is expanding to incorporate recent findings of epigenetic variation of the 5-HT_{2A} receptor gene and specifically methylation sites near the gene promoter region [for review, see Paquette and Marsit (2014)].

Ligands for 5-HT_{2A} receptors have a current untapped potential for the management of cognitive dysfunction. There are well established links between the receptor and the formation of different memory types, including recognition (Morici et al., 2015) and fear memories (Bombardi and Di Giovanni, 2013; Zhang and Stackman, 2015). The latter connects with the considerations of ecstasy and other similar psychotomimetics for the management of post-traumatic stress disorder (Smith et al., 2014; Sessa and Nutt, 2015). There are also very interesting links between the 5-HT_{2A} receptor and impulse control; in animal models, 5-HT_{2A} receptor antagonists consistently reduce measures of impulsivity (Higgins et al., 2003; Winstanley et al., 2004; Fletcher et al., 2007; Winstanley, 2011) and attenuate abuse-related effects of cocaine (Howell and Cunningham, 2015). This raises the possibility that 5-HT_{2A} antagonists/inverse agonists may have utility in the control of addictions and disorders of impulse control more generally.

5-HT_{2A} receptors are required for entry of JC polyomavirus (JCV) into cells (Elphick et al., 2004). JCV causes progressive multifocal leukoencephalopathy, a fatal demyelinating disease. JCV attaches to the sialic acid receptor motif α2,6-linked lactoseries tetrasaccharide c on the cell surface and then undergoes endocytosis with the 5-HT_{2A} receptor (Assetta et al., 2013). 5-HT_{2A} antagonists have been found to inhibit infection of cell by JCV (O'Hara and Atwood, 2008). Furthermore, treatment with mirtazepine, an antidepressant that acts as a 5-HT_{2A} receptor antagonist, has proven beneficial in patients infected with JCV (Verma et al., 2007; O'Hara and Atwood, 2008; Cettomai and McArthur, 2009; Park et al., 2011a).

5-HT_{2A} receptor agonists have ocular hypotensive effects. *R*-(–)-DOI, 5-methoxy-*N*,*N*-dimethyltryptamine, and α-methyl-5-HT have been shown to lower intraocular pressure in a cynomolgus monkey model of ocular hypertension (May et al., 2003). 5-HT_{2A} receptors have been identified in tissues involved in the regulation of aqueous humor dynamics, including ciliary muscle (Sharif et al., 2006a), ciliary epithelium (Inoue-Matsuhisa et al., 2003), and trabecular meshwork (Sharif and Senchyna, 2006; Sharif et al., 2006b). Studies have not completely elucidated the mechanism for the reduction of intraocular pressure by 5-HT_{2A} receptor agonists, but increased uveoscleral outflow may play a role (Gabelt et al., 2005). This effect led to the evaluation of 5-HT_{2A} receptor agonists as potential treatments for glaucoma. To reduce the potential for psychotropic side effects, much of the work to develop 5-HT_{2A} receptor agonists as ocular hypotensive agents has focused on compounds with limited blood-brain barrier permeability. Several 5-HT_{2A} receptor agonists with limited blood-brain barrier permeability have been shown to lower intraocular pressure following topical ocular administration to monkeys, including AL-34662 (1-[(2S)-2-aminopropyl]-1*H*-indazol-6-ol) (Sharif et al., 2007), (8*R*)-1-[(2*S*)-2-aminopropyl]-8,9-dihydro-7*H*-pyrano[2,3-*g*]indazol-8-ol (May et al., 2015), phenylisopropylamines incorporating α-hydroxy or α-methoxy substituents (Glennon et al., 2004), and benzodifuran derivatives (Feng et al., 2007).

Ketanserin can reportedly lower interocular pressure in patients with glaucoma (Costagliola et al., 1993; Mastropasqua et al., 1997). However, the ocular hypotensive effect of ketanserin is attenuated in unilaterally sympathectomized rabbits and therefore is thought to be mediated by a blockade of α_1-adrenoceptors (Chang et al., 1985).

VIII. 5-HT_{2B} Receptor

A. Introduction

5-HT–induced contractions of rat stomach fundus strips were used as a sensitive bioassay for 5-HT long before more specific analytical assays became available (Vane, 1957); however, the 5-HT receptor was only defined more than three decades later as the 5-HT_{2B} receptor. Early pharmacological studies suggested a strong similarity to the 5-HT_{2C} receptor, in line with the high potency of 5-HT and blockade by 5-HT_2 receptor antagonists. However, 5-HT_{2C} receptor mRNA was absent, and molecular cloning identified the new receptor, first in rat and mouse (Foguet et al., 1992a,b; Kursar et al., 1992; Loric et al., 1992; Wainscott et al., 1993) and then in humans (Choi et al., 1994; Kursar et al., 1994; Schmuck et al., 1994; Wainscott et al., 1996). Initially, given the tissue associated primarily with the functional receptor, the receptor was named the 5-HT_{2F} receptor, but it was reclassified subsequently to the 5-HT_{2B} receptor to better fit 5-HT receptor nomenclature. The pharmacological characterization of this receptor in various species confirmed its close relationship to both 5-HT_{2C} and 5-HT_{2A} receptors, as expected from their closely related structural and transductional features. The physiological and pathophysiological functions of the 5-HT_{2B} receptor both in the peripheral and central nervous systems are rather unique.

B. Expression Profile

1. Peripheral Expression. 5-HT_{2B} receptor mRNA and/or protein are detected in the stomach fundus, intestine, liver, kidney, pancreas, spleen, lung, and heart in rats, mice, and humans (Kursar et al., 1992; Choi et al., 1994; Kursar et al., 1994; Choi and Maroteaux, 1996). Functionally active 5-HT_{2B} receptors are present in human uterine smooth muscle (Kelly and Sharif, 2006). The 5-HT_{2B} receptor is also expressed in the vasculature, in various smooth muscle cells (Ullmer et al., 1995), endothelial cells of pig pulmonary arteries (Glusa and Pertz, 2000), human meningeal blood vessels (Schmuck et al., 1996), and rat jugular vein (Ellis et al., 1995). The 5-HT_{2B} receptor is expressed in rat spleen, thymus, and peripheral blood lymphocytes (Stefulj et al., 2000) and rat osteocytes, osteoblasts, and periosteal fibroblasts containing osteoblast precursor cells (Bliziotes et al., 2001; Westbroek et al., 2001). The 5-HT_{2B} receptor gene is expressed in human and rodent anagen, but not telogen skin, in various melanomas and Mel A melanocytes (Slominski et al., 2003, 2004). Finally, the 5-HT_{2B} receptor is expressed in c-kit+ bone marrow cells (Launay et al., 2012). Together, the very heterogeneous expression of the 5-HT_{2B} receptor suggests very diverse physiological and pathophysiological roles.

2. Central Expression. The 5-HT_{2B} receptor is expressed in human cerebral cortex, cerebellar nuclei and their projection areas, lateral septum, dorsal hypothalamus, and medial amygdala (Kursar et al., 1992; Choi et al., 1994; Kursar et al., 1994; Choi and Maroteaux, 1996). The presence of 5-HT_{2B} receptor mRNA was reported in several mammalian brain nuclei, including the dorsal raphe (Bonaventure et al., 2002a). 5-HT_{2B} receptor expression (determined by qPCR) was confirmed in the human frontal, temporal, parietal, and occipital lobes and the olfactory region, cerebellum, diencephalon, hippocampus, thalamus, pituitary gland, pons, medulla oblongata, and nucleus accumbens (Bevilacqua et al., 2010). 5-HT_{2B} receptor mRNA was reported in rat cultured astrocytes (Sanden et al., 2000; Osredkar and Kržan, 2009). Microglial expression of 5-HT_{2B} receptors was documented in primary cultured and acutely isolated adult microglia, using two-photon microscopy on brain slices (Kolodziejczak et al., 2015) and patch-clamp studies in cultured microglia (Krabbe et al., 2012), suggesting 5-HT_{2B} receptor may mediate modulation of microglial functions such as phagocytosis and migration, synaptogenesis, and neuronal death.

The 5-HT_{2B} receptor modulates the release of rat growth hormone (GH) in the pituitary (Papageorgiou and Denef, 2007). The 5-HT_{2B} receptor is expressed in the spinal cord (Helton et al., 1994; Holohean and Hackman, 2004), the rat organ of Corti lateral wall, and spiral ganglion subfractions (Oh et al., 1999) and is upregulated in ageing mice cochlea (Tadros et al., 2007). 5-HT_{2B} receptor mRNA is expressed predominantly in the human retina, ciliary body, ciliary epithelium, choroid, conjunctiva, and iris. 5-HT_{2B} receptor mRNA was also documented in optic nerve tissue of human donor trabecular meshwork cells (Sharif and Senchyna, 2006).

C. Post-translational Modifications and Impact

1. Gene Structure. The 5-HT_{2B} receptor gene is composed of four Exons, including one 5′ noncoding exon and three coding exons in all vertebrates that have been sequenced.

2. Gene Regulation. The transcriptional regulation of the 5-HT_{2B} receptor gene is not well understood. In breast tumors, the c-Myc transformation induces an increased 5-HT_{2B} receptor expression (Pai et al., 2009). In human umbilical endothelial cells, Wnt2 downstream targets the 5-HT_{2B} receptor gene (Klein et al., 2009). The 5-HT_{2B} receptor expression is downregulated via nuclear factor-κB (NF-κB) (RelAp65-p52) in C-reactive protein–stimulated pulmonary arterial endothelial cells (Wynants et al., 2013). In pulmonary

artery smooth muscle cells, 5-HT induces 5-HT_{2B} receptor mRNA expression, which is inhibited by peroxisome proliferator–activated receptor (PPAR)γ activation and suggests the suppression of AP-1 activity (Liu et al., 2012b; Maroteaux, 2013). The presence of retinoic acid response elements in the 5-HT_{2B} receptor promoter suggests a negative regulatory relationship between retinoic acid and 5-HT signaling at sites of epitheliomesenchymal interaction (Bhasin et al., 2004).

3. Receptor Isoforms. Only a few studies have investigated putative splice variants in the 5-HT_{2B} receptor. In puffer fish, 5-HT_{2B} receptor splicing variants have been identified (De Lucchini et al., 2001). Splice variant 1 contains a 136-bp deletion that eliminates a portion of exon 2 by alternative splice sites located within transmembrane regions I and II, which results in a premature stop codon to produce a very short truncated 31-amino-acid protein. Splice variant 2 contains a 201-bp deletion because of an exon-skipping mechanism that eliminates exon 3 (which is also found in human and mice). The resulting RNA retains the same open reading frame. Splice variant 3 contains a 19-bp deletion, probably by an alternative 5′ splice site upstream of the canonical 5′ splice site of intron C; this leads to a frame shift and a premature termination codon, which was also found in human and mice. This short variant results in a putative 177-amino-acid protein, with 28 specific residues at the carboxyl terminus. The functions of these splice variants remain to be defined. Finally, an exon-skipping mechanism that eliminates exon 3 was found in fish, humans, and rodents; this leads to a truncated receptor containing only the first transmembrane domain.

D. Protein Structure

The 5-HT_{2B} receptor displays the characteristic structure of a GPCR receptor, with a relatively long N-terminus of about 55 amino acids. The human 5-HT_{2B} receptor has 481 amino acids (479 amino acids in rat or mouse), with 79% and 82% homology for human versus rat and mouse, respectively. The 5-HT_{2B} receptor N-terminus may act as a negative modulator, affecting both constitutive and agonist-stimulated activity (Belmer et al., 2014).

The ergotamine-bound 5-HT_{2B} receptor crystal structure exhibits some conformational features of both the active and inactive states: an active-like state in the helix VII conformation but only partial changes in helix VI, which mirror the strong β-arrestin bias of ergotamine seen in functional assays (Wacker et al., 2013; Wang et al., 2013). A structural explanation for the distinct conformational features and the biased pharmacology of ergotamine can be seen in the extracellular loop 2 (ECL2) junction with helix V, E212-R213-F214 forming an additional helical turn stabilized by a structured water molecule at the extracellular tip of helix V. The segment of ECL2 connecting helices III and V via the conserved disulfide bond is, therefore, shortened and creates a conformational constraint on the extracellular tip of helix V (Martí-Solano et al., 2014). However, this structured water molecule involved in ECL2 junction with helix V has been challenged, as differential interactions of ergotamine with the top of helices V and VI could determine the rotational freedom of helix VI (Liu et al., 2013a). For more discussion of the crystal structure, see *XVI. A. 5-HT GPCRs*.

E. Heteromeric Receptor Associations

In cardiac fibroblasts, angiotensin AT1 receptors and 5-HT_{2B} receptors, which share common signaling pathways, could exist in heterodimeric complexes as shown by coimmunolocalization and a pull-down assay (Jaffre et al., 2009), but experimental confirmation is lacking.

F. Pharmacology

1. Agonists. Biased agonism is evident with a range of drugs that impact the 5-HT_{2B} receptor, and the influence of this phenomenon on the pharmacological profile of agonists can be profound (see, for example, Huang et al., 2009). Hence, when defining the action of agonists at the 5-HT_{2B} receptor—at least in terms of their potency and efficacy—the particular readout (e.g., $[Ca^{2+}]_i$, arrestin, ERK, IP_3) and the nature of the receptor preparation needs to be taken into account. Against this background, the pharmacology of agonists will be described.

BW723C86, 1-methyl-2-[5-(2-thienylmethoxy)-1H-indole-3-yl] ethylamine hydrochloride, has 10- and 100-fold selectivity for the human 5-HT_{2B} receptor over the human 5-HT_{2C} and 5-HT_{2A} receptors, respectively. (recommended use: $\leq$100 nM concentration or $\leq$3 mg/kg i.p. in rodents; Porter et al., 1999; Jerman et al., 2001; Knight et al., 2004; Cussac et al., 2008). α-Methyl-5-HT is a full agonist with high potency for the 5-HT_{2B} receptor (pEC_{50} = 8.4) and lower potency for both 5-HT_{2C} and 5-HT_{2A} receptors. 5-Methoxytryptamine is also 25- and 400-fold selective over the 5-HT_{2A} and 5-HT_{2C} receptors, respectively. Nordexfenfluramine (metabolite of dexfenfluramine), methylergonovine (metabolite of methysergide), and Ro 60-0175 (2(S)-1-(6-chloro-5-fluoro-1H-indol-1-yl)-2-propanamine fumarate) are all somewhat preferential 5-HT_{2B} receptor agonists with about 10-fold selectivity over 5-HT_{2C} receptor.

The 5-HT_{2B} receptor displays high affinity to 5-HT (Kd ~10 nM) and many nonselective 5-HT_2 receptor active compounds, including some metabolites of therapeutics and drugs of abuse. Such agonists include MDA (3,4-methylene dioxyamphetamine-MDA, a metabolite of 3,4-methylenedioxy methamphetamine-MDMA) (Setola et al., 2003), MDMA ("ecstasy") itself, tryptamine, and LSD. DOI is a nearly full agonist at 5-HT_{2B} receptors but with similar affinity to 5-HT_{2A} and 5-HT_{2B} receptors (Porter et al., 1999; Jerman et al., 2001; Knight et al., 2004; Cussac et al., 2008).

Many substances known as "legal highs" display notable affinity for 5-HT_{2B} receptors, including 5-APB, commonly "marketed" as "benzofury" (K_i = 14 nM) and 6-APB (K_i = 3.7 nM), and 5-iodo-aminoindane (K_i = 70 nM). 5-APB and 6-APB act as potent (i.e., nanomolar EC_{50} values) full agonists at 5-HT_{2B} receptors (Iversen et al., 2013; Rickli et al., 2015). 5-APB contracts the rat stomach fundus and is antagonized by the 5-HT_{2B} receptor antagonist, RS127445 (Dawson et al., 2014). Other such drugs show submicromolar affinities for the 5-HT_{2B} receptor (mephedrone, naphyrone, 1-naphyrone, and methylenedioxy-aminotetralin). Indeed, there is a correlation in a series of phenyliso-propylamines between hallucinogenic activity and affinity for the 5-HT_{2B} receptor (Nelson et al., 1999), although 5-HT_{2A} receptor agonism is still also considered to play a major role in "psychedelics," such as LSD or psyloscibine. Activation of the 5-HT_{2B} receptor appears to play a key role in the behavioral stimulant and 5-HT releasing effects of MDMA (Doly et al., 2008) and in the reinforcing effects of MDMA in mice (Doly et al., 2009).

2. Antagonists. The first highly 5-HT_{2B} selective antagonist is LY266097, 1-(2-chloro-3,4- dimethoxybenzyl)-6-methyl-1,2,3,4-tetrahydro-9Hpyrido [3,4-b]indole hydrochloride, with a pK_i of 9.7 for the human cloned 5-HT_{2B} receptor and a 100-fold greater selectivity over human 5-HT_{2C} and 5-HT_{2A} receptor binding sites (recommended use: 20 nM concentration in vitro or 0.5 mg/kg i.p. in rodents; Audia et al., 1996). SB204741, N-(1-methyl-5-indolyl)-N'-(3-methyl-5-isothiazolyl)urea, is another selective 5-HT_{2B} receptor antagonist with approximately 100-fold selectivity over the 5-HT_{2C} and 5-HT_{2A} sites but with rather low potency (K_i around 100 nM) (recommended use: 500 nM concentration in vitro or 10 mg/kg i.p. in rodents; Bonhaus et al., 1995). The tetrahydro-β-carboline, LY272015 (6-chloro-5-methyl-N-(5-quinolinyl)-2,3-dihydro-1H-indole-1-carboxamide) is also a fairly selective and highly potent antagonist (recommended use: 50 nM concentration in vitro or 1.0 mg/kg i.p. in rodents; Cohen et al., 1996). RS127445, 2-amino-4-(4-fluoronaphth-1-yl)-6-isopropyl-pyrimidine, has subnanomolar affinity for the 5-HT_{2B} receptor (pK_i = 9.5) and 1000-fold selectivity compared with numerous other receptors and ion channels, and it appears as the most selective, high-affinity 5-HT_{2B} receptor antagonist suitable now (Bonhaus et al., 1999; recommended use: 20 nM concentration or 0.25 mg/kg i.p. in rodents). The methoxythioxanthene BF-1 is a highly selective and potent 5-HT_{2B} receptor antagonist lacking high affinities for 5-HT_{1A}, 5-HT_{2A}, 5-HT_{2C}, histamine H_1, dopamine D_1, and D_2 as well as muscarinic M_1 and M_2 receptors (Schmitz et al., 2015). S33526, 6-chloro-2,3,4,9-tetrahydro-1H-b-carbolin-1-yl)-phenyl-acetic acid ethyl ester, is a high-affinity and relatively selective antagonist at 5-HT_{2B} receptors (Cussac et al., 2002). SB215505, 6-chloro-5-methyl-N-(5-quinolinyl)-2,3-dihydro-1H-indole-1-carboxamide, behaves as a high-affinity and preferential inverse agonist at 5-HT_{2B} receptors. SB206553, 5-methyl-N-(3-pyridyl)-1,2,3,5-tetrahydrobenzo[1,2-b:4,5-b']dipyrrole-1-carboxamide, is a mixed 5-HT_{2C}/5-HT_{2B} receptor inverse agonist with 50- to 100-fold lower affinity for the 5-HT_{2A} and other sites.

Nonselective 5-HT_2 receptor antagonists such as ritanserin and metergoline antagonize 5-HT_{2B} receptor–mediated effects. Furthermore, the α_2 adrenergic receptor antagonists yohimbine and rauwolscine are potent 5-HT_{2B} antagonists, with low affinity for the 5-HT_{2C} and 5-HT_{2A} receptors. Atypical antipsychotics have also fairly high affinity for 5-HT_{2B} receptors, including clozapine, asenapine, or cariprazine (Wainscott et al., 1996; Millan et al., 2003; Shahid et al., 2009; Kiss et al., 2010). Aripiprazole (OPC-14597) is a novel atypical antipsychotic, with high antagonist affinity (IC_{50} = 11 nM) for the human 5-HT_{2B} receptor (Shapiro et al., 2003).

3. Allosteric Modulators. No selective 5-HT_{2B} allosteric modulator has been definitely identified. In the crystal, ergotamine binds to two distinct sites at the 5-HT_{2B} receptor, the orthosteric site, where the indole nucleus of ergotamine resides, and the "extended" binding site, to which the tripeptide portion of the ergoline binds. This potential allosteric site is also present in the muscarinic M2 receptor at the same extracellular region. The similarities in both the M2 and 5-HT_{2B} receptors suggest that the location of the extracellular allosteric site for class A GPCRs is rather similar and conserved; these common features suggest that ergotamine and other ergolines may function as "bitopic" ligands, acting at both the orthosteric and the putative extracellular allosteric site in the 5-HT_{2B} receptor. It is thought that a sodium ion allosterically modulates the binding pocket to reduce G protein signaling, thus favoring β-arrestin recruitment (McCorvy and Roth, 2015).

G. Transduction System(s)

1. Transfected Cells. 5-HT_{2B} receptors expressed in mouse fibroblast L-cells stimulate GTPase activity and inositol 1,4,5-triphosphate production upon agonist stimulation. This GTPase activation is mediated by $G\alpha_{q/11}$ but not by $G\alpha s$ or $G\alpha i$. The GTPase activation was also blocked by anti–β1-4 or anti-γ2 subunit antibodies. The 5-HT_{2B} receptor couples to phospholipase A2 (PLA2)-mediated release of arachidonic acid (Tournois et al., 1998). In addition, stimulation of the 5-HT_{2B} receptor triggers intracellular cyclic guanosine monophosphate (cGMP) production through dual activation of constitutive nitric-oxide synthase (cNOS) and inducible NOS. The group I PDZ motif at the carboxy terminus of the 5-HT_{2B} receptor is required for cNOS transduction pathways, whereas inducible NOS stimulation is $G\alpha$13-dependent (Manivet et al., 2000). The 5-HT_{2B} receptor shares the C-terminal

E-X-V/I-S-X-V sequence with the 5-HT_{2C} receptors and binds MUPP1-PDZ domains in vitro (Becamel et al., 2001). Agonist-induced stimulation of the 5-HT_{2B} receptor promotes rapid and transient activation of the proto-oncogene product $p21^{ras}$, as measured by an increase in GTP-bound Ras (Launay et al., 1996). 5-HT_{2B} receptor stimulation activates the MAPKs $p42^{mapk}$/$p44^{mapk}$ as well as ERK2/ERK1. It results in the formation of foci and to the formation of tumors from these foci in nude mice (Launay et al., 1996). The 5-HT_{2B} receptor–dependent cell-cycle progression happens through retinoblastoma protein hyperphosphorylation and the activation of both cyclin D1/cdk4 and cyclin E/cdk2 kinases. The induction of cyclin D1, but not that of cyclin E expression, is under MAPK control, indicating an independent regulation of these two cyclins in 5-HT_{2B} receptor–induced mitogenesis. Similarly, platelet-derived growth factor receptor (PDGFR) kinase activity is essential for 5-HT_{2B}-triggered MAPK/cyclin D1, but not cyclin E, signaling pathways. 5-HT_{2B} receptor activation also increases activity of the Src family kinases c-Src, Fyn, and c-Yes. Strikingly, c-Src, but not Fyn or c-Yes, is the crucial link between the Gq protein–coupled 5-HT_{2B} receptor and the cell-cycle regulators (Nebigil et al., 2000b). Inhibition of c-Src activity is sufficient to abolish 5-HT–induced PDGFR tyrosine kinase phosphorylation and MAPK activation, cyclin D1 and cyclin E expression levels, and thymidine incorporation. Thus, c-Src activation by the 5-HT_{2B} receptor controls cyclin E induction and, in concert with PDGFR, also induces cyclin D1 expression via the MAPK/ERK pathway (Nebigil et al., 2000b). The 5-HT_{2B} signal transduction pathways are thus quite diverse and are similar to those of 5-$HT_{2A/2C}$ receptors. On the other hand, the NOS pathway seems to be 5-HT_{2B} receptor–specific.

2. Primary Cell Cultures. In cultivated cardiac fibroblasts, angiotensin II (AngII)- or 5-HT–dependent cytokine release is critical for the expression of HB-EGF and Src activity via endogenous AT1 and 5-HT_{2B} receptors (Jaffre et al., 2009). Matrix metalloproteinases (MMPs) are responsible for HB-EGF shedding and subsequent EGF-receptor transactivation induced by AngII or 5-HT. Tumor necrosis factor-α (TNF-α)–converting enzyme controls HB-EGF shedding in fibroblasts and is directly regulated by 5-HT_{2B} receptors (Pietri et al., 2005). Blockade of one of the two receptors prevents cytokine release induced by the other receptor (Jaffre et al., 2009). These findings also indicate that AT1 and 5-HT_{2B} receptors share common EGF receptor–dependent signaling pathways in adult cardiac fibroblasts, and the two receptors were shown to interact in a common cell compartment. Together, the data support AT1 and 5-HT_{2B} receptors exist as heterodimers that may play a key role in receptor maturation and trafficking to the plasma membrane and/or signaling (Bulenger et al., 2005) to drive common signaling regulating hypertrophic factors in the heart (Jaffre et al., 2009).

5-HT_{2B} receptor stimulation in hepatic stellate cells (HSC) activates the expression of TGFβ1 (a powerful suppressor of hepatocyte proliferation) via ERK/JunD signaling. 5-HT_{2B} receptor antagonists decrease the mRNA levels of TGFβ1, connective growth factor, plasminogen activator inhibitor-1, Smad-3, and JunD in lung and skin fibroblasts (Dees et al., 2011). 5-HT_{2B} receptor activation leads to sustained phosphorylation of two downstream targets of mTOR, p70S6K and 4E-BP1, thereby facilitating survival and inhibiting autophagy of hepatocellular carcinomas (Soll et al., 2010). The 5-HT_{2B} receptor protects newborn postmitotic cardiomyocytes against serum deprivation–induced apoptosis as manifested by DNA fragmentation, nuclear chromatin condensation, and terminal deoxynucleotidyl transferase dUTP nick end labeling. 5-HT prevents cytochrome c release and caspase-9 and -3 activation after serum deprivation via crosstalk between phosphatidylinositol-3 kinase (PI3K)/Akt and ERK1/2 signaling pathways. 5-HT_{2B} receptor–activated ERK kinases inhibit Bax expression induced by serum deprivation. 5-HT activates NF-κB via PI3K/Akt required for the regulation of the mitochondrial adenine nucleotide translocator and mitochondrial permeability. Thus, 5-HT via the 5-HT_{2B} receptor is a novel survival factor targeting mitochondria (Nebigil et al., 2003). Interestingly, NF-κB regulation by 5-HT_{2B} receptors is confirmed in a large screen for genes regulating NF-κB and the MAPK pathways (Matsuda et al., 2003).

Primary osteoblasts from mutant 5-HT_{2B} receptor KO mice show reduced proliferation and delayed differentiation; calcium incorporation is markedly reduced in osteoblasts after 5-HT_{2B} receptor inactivation (by genetic invalidation or by pharmacological inhibition; Collet et al., 2008). A functional link between the 5-HT_{2B} receptor and the activity of the tissue-nonspecific alkaline phosphatase (TNAP) was established in an osteoprogenitor C1 cell line (Baudry et al., 2010a). During osteogenic differentiation, both 5-HT_{2B} receptor and TNAP mRNA translations are delayed with respect to extracellular matrix deposition. Once the receptor is expressed, it constitutively controls TNAP activity at a post-translational level along the entire period of mineral deposition. The lack of 5-HT_{2B} receptors is associated with a 10-fold overproduction of prostacyclin in osteoblast primary cultures. A specific prostacyclin synthase (CYP8A1) inhibitor (U51605) totally rescued osteoblast aggregation and matrix mineralization in 5-HT_{2B} receptor KO osteoblasts without any effect on WT osteoblasts. Prostacyclin is the endogenous ligand of the nuclear receptor PPAR-β/δ, and its inhibition in 5-HT_{2B} KO cells totally rescued the alkaline phosphatase *TNAP* and osteopontin *SPP1* mRNA levels, cell-cell adhesion, and matrix mineralization. The absence of 5-HT_{2B} receptors leads to the overproduction of prostacyclin, inhibiting osteoblast differentiation because of PPAR-β/δ–dependent target regulation and defective

cell-cell adhesion and matrix mineralization (Chabbi-Achengli et al., 2013), supporting a physiologic negative control of prostacyclin synthase by 5-HT_{2B} receptors. Thus, endogenously expressed 5-HT_{2B} receptors can modulate various transduction pathways, including Src, MMPs, and PLA2 activities in a cell type–dependent manner.

H. Regulatory Mechanisms

1. Internalization. In transfected cells, prior exposure to 5-HT results in a rapid and considerable (up to 80%) 5-HT_{2B} receptor desensitization (Porter et al., 2001). Internalization of 5-HT_{2B} receptors is caveolin1-independent and clathrin- and β-arrestin2–dependent (Janoshazi et al., 2007).

Some ergot derivatives are "slow" 5-HT_{2B} receptor binders, with very slow association and dissociation rates. The compounds have apparent lower potency to increase intracellular concentrations of calcium ions relative to inositol phosphate accumulation assays. Similarly, the potency of ergolines to activate ERK1/2 is highly time-dependent. In addition, a number of ergot derivatives produce "wash-resistant" 5-HT_{2B} receptor signaling that persists for hours without appreciable loss of potency, which is not explained simply by slow receptor-dissociation kinetics. Thus, this persistent signaling has been proposed to originate from internalized or sequestered receptors (Unett et al., 2013). The 5-HT_{2B} receptor crystal structure (Huang et al., 2009) reveals an intermediate state of activation stabilized by the extracellular-facing tripeptide portion of ergotamine, which likely drives β-arrestin bias and is not seen in unbiased ligands such as 5-HT itself. Thus, the long duration of action of some ergolines may be explained by a combination of very slow kinetics at the receptor, coupled with persistent intracellular signaling.

2. Interacting Proteins. Proteins known to interact with the 5-HT_{2B} receptor include constitutive and inducible NOS; Gαq, Gα11, and Gα13, involved in signaling of the receptor; and MUPP1, a multivalent PDZ scaffolding protein. (Becamel et al., 2001).

For more details on proteins that interact with the 5-HT_{2B} receptor, see *XVII. B. 4. 5-HT_{2B} Receptor*.

I. Function at Cellular, Tissue, and In Vivo Level

1. Hematopoiesis. 5-HT promotes megakaryocyte (MK) proliferation and reduces cell apoptosis via activation of the 5-HT_{2B} receptor and Akt pathway (Liu and Yang, 2006). 5-HT increases proplatelet-bearing MKs and polymerizes actin via ERK1/2 (Ye et al., 2014). *Tph1*$^{-/-}$ mice are deficient in peripheral 5-HT and display features of ineffective erythropoiesis. The central event starts in the bone marrow where the absence of 5-HT inhibits the terminal differentiation of erythroid precursors expressing 5-HT_{2A} and 5-HT_{2B} receptors. In addition, red blood cells from 5-HT–deficient mice are more sensitive to macrophage phagocytosis and have a shortened in vivo half-life (Amireault et al., 2011). In addition, the 5-HT_{2B} receptor is expressed in c-kit+ bone marrow cells (Launay et al., 2012). The 5-HT_{2B} receptor antagonist RS127445 decreases colony-forming capacity, with inhibition of both early stem/progenitors and erythroid burst-forming unit formation attributed to a reduction of cell proliferation and/or an apoptotic effect. By contrast, 5-HT significantly enhances the expansion of CD34+ cells to early stem/progenitors and committed progenitors (erythroid burst-forming units) (Yang et al., 2007).

In human macrophages, 5-HT inhibits the LPS-induced release of proinflammatory cytokines to upregulate the expression of M2 polarization–associated genes and to reduce the expression of M1-associated genes. 5-HT_{2B} receptors mediate the pro-M2 skewing effect of 5-HT. Blockade of this receptor during in vitro monocyte-to-macrophage differentiation preferentially modulates the acquisition of M2 polarization markers. 5-HT_{2B} receptor mRNA is preferentially expressed by anti-inflammatory M2 (macrophage colony-stimulating factor) macrophages and is detected in vivo in liver Kupffer cells and in tumor-associated macrophages (de Las Casas-Engel et al., 2013). 5-HT_{2B} receptor expression is found in postnatal microglia, suggesting that 5-HT participates in microglial functions (Kolodziejczak et al., 2015). 5-HT_{2B} receptor mRNA expression is evident in spleen, thymus, and peripheral blood lymphocytes (Stefulj et al., 2000). Immature dendritic cells express 5-HT_{2B} receptor mRNA, and 5-HT_{2B} receptor stimulation induces intracellular Ca^{2+} mobilization in immature, but not mature, dendritic cells. Thus, 5-HT stimulates, in a maturation-dependent manner, different signaling pathways in dendritic cells (Idzko et al., 2004).

A proper balance between different T-helper (Th) cell subsets is necessary for normal functioning of the adaptive immune system. Th cells (from human umbilical cord blood) differentiated in vitro into Th1 or Th2 cells reveal the latter express 5-HT_{2B} receptor mRNA (Aijö et al., 2012). In gene expression profiles during human CD41 T-cell differentiation, 5-HT_{2B} receptor mRNA was found to be SP4-specific (~10-fold) among the 16 transcripts expressed in SP4 thymocytes at levels threefold or higher than in any other isolated T-cell subpopulation (Lee et al., 2004b).

Treatment with aggregated (1–40 or 1–42) and oligomeric (1–42) amyloid β (Aβ, found in Alzheimer disease) promoted differentiation of bone marrow–derived mesenchymal stem cells without toxic effects. The effect of Aβ was shown to be mediated by the neuropeptide Y1 receptor and the 5-HT_{2B} receptor via PI3K-dependent activation of the MAPK/ERK1/2 pathway (Jin et al., 2009). Thus, the 5-HT_{2B} receptor, among others, mediates the balance among various hematopoietic lineages.

2. Pancreas. A strong lactogen-dependent upregulation of 5-HT biosynthesis takes place in a subpopulation of

mouse islet β-cells during pregnancy (Schraenen et al., 2010). Pancreatic islet cells express the genes encoding all of the products necessary for synthesizing, packaging, and secreting 5-HT, including both isoforms of the 5-HT synthetic enzyme tryptophan hydroxylase (TPH) and the archetypal 5-HT transcription factor Pet1. In β-cells, Pet1 can bind to the 5-HT–relevant genes but also to a conserved insulin gene regulatory element. Mice lacking Pet1 display reduced insulin production and secretion and impaired glucose tolerance (Ohta et al., 2011). Inhibition of 5-HT synthesis blocks β-cell expansion and induced glucose intolerance in pregnant mice without affecting insulin sensitivity. Expression of the 5-HT_{2B} receptor in maternal islets has been reported to increase during pregnancy and to normalize just before parturition. Blocking 5-HT_{2B} receptor signaling in pregnant mice may also block β-cell expansion and cause glucose intolerance (Kim et al., 2010).

3. Adipocytes. By inhibiting 5-HT_{2B} receptor signaling during adipogenesis using RS127445, an increased fat accumulation was observed similar to the knockdown phenotype (Söhle et al., 2012). In adipocytes, 5-HT_{2B} receptors favor lipolysis by increasing phosphorylation and activity of hormone-sensitive lipase (Sumara et al., 2012).

4. Cardiovascular and Pulmonary Systems. 5-HT_{2B} receptor inactivation in mice leads to partial embryonic lethality caused by major defects in heart development (Monassier et al., 2010). Neonates exhibit a second wave of partial lethality because of cardiac dilation resulting from contractility deficits and structural deficits at the intercellular junctions between cardiomyocytes. Echocardiography and electrocardiography studies in animals that live past the first week and survive until adulthood confirm the presence of left-ventricular dilation and decreased systolic function. 5-HT, via the 5-HT_{2B} receptor, regulates heart differentiation and proliferation during development as well as cardiac structure and function in adults (Nebigil et al., 2000a). The 5-HT_{2B} receptor is functionally coupled to ROS synthesis through NADPH oxidase (NOX) stimulation in 1C11 cells (Schneider et al., 2006) and in angiotensin II and isoproterenol-induced cardiac hypertrophy (Monassier et al., 2008). In human atrial myocytes, 5-HT reduces the amplitude of L-type calcium currents and affects the strength of gap junctional intercellular communication, which is markedly reduced by blocking receptors, showing that activation of 5-HT_{2B} receptors inhibit gap junctional intercellular communication (Derangeon et al., 2010). Upon pulmonary artery banding, the 5-HT_{2B} receptor antagonist SB204741 reduces right-ventricular fibrosis and improves heart function in mice (Janssen et al., 2015).

A model in which 5-HT_{2B} receptor signaling promotes cardiac hypertrophy by stimulating calcineurin/nuclear factor of activated T cells signaling suggests the recruitment of histone acetyl transferases to regulatory regions of nuclear factor of activated T-cells target genes. 5-HT_{2B} receptor agonist–induced hypertrophy of cardiac muscle cells results from a signaling pathway involving calcineurin and a kinase-dependent mechanism that inactivates class II histone deacetylases (HDAC), which act as repressors of cardiac growth (Bush et al., 2004). Because it also stimulates nuclear export of class II HDACs, myocyte enhancer factor-2 protein may play a role in the mechanism by which 5-HT_{2B} receptor signaling triggers cardiac remodeling (McKinsey and Olson, 2005). A cDNA encoding the 5-HT_{2B} receptor was found in a screen for genes encoding HDAC5 modulators, and the ability of 5-HT_{2B} receptors to promote HDAC5 phosphorylation and cardiomyocyte hypertrophy was confirmed (McKinsey and Olson, 2005). The 5-HT_{2B} receptor–triggered intracellular calcium ion release and PKC activation accounts, at least in part, for the overexpressed receptor-induced HDAC5 phosphorylation (Chang et al., 2005).

5. Endothelial Cells. In human pulmonary artery endothelial cells, 5-HT_{2B} receptors stimulate calcium ion release from intracellular stores (Ullmer et al., 1996a). 5-HT_{2B} receptors mediate the endothelium-dependent relaxation of rat jugular vein (Ellis et al., 1995) and pig pulmonary artery (Glusa and Pertz, 2000). Activation of 5-HT_{2B}/5-HT_{1B} receptors stimulates NO production in human coronary artery endothelial cells (Ishida et al., 1998). Compared with nonstimulated pulmonary artery endothelial cells, C-reactive protein–stimulated cells show downregulation of 5-HT_{2B} receptor by 25%, of inhibitor of NF-κB kinase subunit epsilon by 30%, and of toll-like receptor-4 and -6 by 18% and 39%, respectively (Wynants et al., 2013). A cardioprotective function of the 5-HT_{2B} receptor in an integrated model of heart failure with preserved ejection fraction can be explained by a contribution of the endothelial 5-HT_{2B} receptors to coronary vasodilatation (Ayme-Dietrich et al., 2015).

6. Aorta. In normotensive rats, 5-HT–induced contraction of the aorta is primarily 5-HT_{2A} receptor–dependent; however, in hypertensive rats, it is mediated by both 5-HT_{2A} and 5-HT_{2B} receptors. The endothelium-denuded isolated superior mesenteric artery of hypertensive (DOCA-salt) rat displays a marked increase in maximum arterial contraction to 5-HT_{2B} receptor agonists when compared with control rats, confirming that the 5-HT_{2B} receptor plays a greater role in 5-HT–induced contraction in arteries from hypertensive rats (Watts and Fink, 1999; Banes and Watts, 2003).

7. Liver. 5-HT is a potent growth factor for liver development and regeneration. The expression of both 5-HT_{2A} and 5-HT_{2B} receptors in the liver increases following hepatectomy. 5-HT_2 receptor inhibition by ketanserin blocks liver regeneration when administrated close to the G1/S transition point, suggesting 5-HT as a cofactor for DNA synthesis (Papadimas et al., 2006). In $Tph1^{-/-}$ mice, the failure of liver regeneration

is rescued by reloading 5-HT–free platelets with a 5-HT precursor molecule (Lesurtel et al., 2006). Elderly mice have decreased ability of the liver to restore normal volume after partial hepatectomy. The 5-HT_2 receptor agonist DOI reverses the age-related pseudocapillarization of old liver and improves hepatosinusoidal blood flow (Furrer et al., 2011); it also enhances hepatocyte proliferation after liver transplantation in mice. 5-HT_{2B} receptor activation significantly improves survival in recipients of a small, otherwise nonviable graft by enhancing liver regeneration and hepatic microcirculation, thereby reducing ischemia/reperfusion injury (Tian et al., 2011). 5-HT protects the liver in an IL-6–independent manner. The protective effects of DOI is lost in animals treated with SB206553, a 5-$HT_{2B/2C}$ receptor antagonist. DOI may thus preserve microcirculation and accelerate liver regeneration via 5-HT_{2B} receptors, thus preventing the liver parenchyma from further injury (Tian et al., 2011). However, in a pathophysiological setting, the regenerative influence of 5-HT acting through 5-HT_{2A} receptors on hepatocytes may be subjected to opposite antiregenerative effects arising from 5-HT acting through 5-HT_{2B} receptors in hepatic stellate cells (Ebrahimkhani et al., 2011). In hepatocytes, signaling through 5-HT_{2B} receptors also promotes gluconeogenesis (Sumara et al., 2012).

8. Gut. The 5-HT_{2B} receptor was initially characterized as the receptor contracting the rat stomach fundus (Vane, 1957). The 5-HT_{2B} receptor is expressed by smooth muscles in the small intestine, the stomach, and by enteric neurons. 5-HT, stimulating 5-HT_{2B} receptors, affects the fate of the large subset of enteric neurons that arises after the development of endogenous sources of 5-HT (Fiorica-Howells et al., 2000). High levels of both 5-HT_{2B} receptor mRNA and protein are found predominantly in the muscle layers and in the myenteric nerve plexus throughout the colon, where they cause neuronally mediated contractions of longitudinal muscle (Borman et al., 2002) and tonically regulate colonic motility (Bassil et al., 2009).

Ghrelin, an orexigenic peptide present in the stomach, has gastroprokinetic properties. In vivo, the ghrelin receptor antagonist D-Lys(3)-GHRP-6 reduces food intake and delays gastric emptying in rodents. D-Lys(3)-GHRP-6 contracts stomach strips, an effects blocked by methysergide and yohimbine, suggesting an interaction with 5-HT_{2B} receptors (Depoortere et al., 2006).

Normal gastrointestinal (GI) motility requires functional interstitial cells of Cajal (ICC) that proliferate in adult mice. 5-HT_{2B} receptor activation increases the proliferation of ICC in vivo. On the other hand, lack of 5-HT_{2B} receptor signaling reduces the density of ICC networks in mature mice. Targeting 5-HT_{2B} receptors may protect ICC networks from injury and/or help repair them after injury (Tharayil et al., 2010).

9. Central Autonomic Nervous System. 5-HT_{2B} and 5-HT_{2A} receptors are expressed in pontomedullary respiratory motor centers. They are colocalized in the Kolliker-Fuse and parabrachial regions of the pons and in the Bötzinger and pre-Bötzinger complex of the ventral medulla with a stronger expression of 5-HT_{2A} receptors in all regions. BW723C86 applied in the pre-Bötzinger complex increases respiratory frequency and is blocked by the 5-HT_{2B} receptor–selective antagonist, LY272015. Hence, endogenous 5-HT appears to support tonic action on respiratory rhythm generation via 5-HT_{2B} receptors. However, respiratory activity is unaffected in 5-HT_{2B} receptor–deficient mice; BW723C86 and LY272015 had no effects, whereas ketanserin blocked rhythmic activity. Thus, endogenous 5-HT_{2B} receptor activation has a stimulatory role at the pre-Bötzinger complex and hypoglossal motoneurons that can be substituted by 5-HT_{2A} receptors in the absence of 5-HT_{2B} receptors. The presence of functional 5-HT_{2B} receptors in the neonatal medullary breathing center suggests a potential convergent regulatory role of 5-HT_{2B} and 5-HT_{2A} receptors on the central respiratory network (Günther et al., 2006). When 5-HT_{2A} and 5-HT_{2B} receptor agonists are applied concurrently, there is a frequency increase that exceeds the effects of either agonist alone. This is an expected outcome if frequency-controlling neurons are preferred targets for 5-HT_{2B} receptor modulation in the pontomedullary respiratory compartment (Niebert et al., 2011).

Phrenic motor neurons express 5-HT_{2B} receptors, and acute intermittent hypoxia induces phrenic long-term facilitation via spinal 5-HT_2 receptor activation and NOX activity. Pretreatment with NOX inhibitors blocks 5-HT_{2B} receptor–induced phrenic motor facilitation (Macfarlane et al., 2011). Episodic spinal 5-HT receptor activation alone, without changes in oxygenation, is sufficient to elicit NOX-dependent phrenic motor facilitation. Intrathecal BW723C86 elicits progressive and sustained phrenic motor facilitation, blocked by the 5-HT_{2B} receptor antagonist SB206553. Spinal neuronal NOS (nNOS) activity is necessary for acute intermittent hypoxia-induced phrenic long-term facilitation (Macfarlane et al., 2014). Thus, episodic spinal NO elicits phrenic long-term facilitation via 5-HT_{2B} receptor activation and NOX-derived ROS formation.

10. Peripheral Autonomic Nervous System. Heart rate control originates in parasympathetic preganglionic cardiac vagal neurons in the nucleus ambiguus. 5-HT_2 receptors modulate spontaneous and respiratory-evoked GABAergic neurotransmission to cardioinhibitory vagal neurons within the nucleus ambiguus as well as rhythmic fictive inspiratory-related activity. Neurons in the rat nucleus tractus solitarius are affected differently by 5-HT_2 receptor ligands regarding their vagal postsynaptic location, the type of cardiopulmonary afferent they receive, and the nature of the 5-HT_2 receptor. 5-HT_{2B} receptors are excitatory, whereas 5-HT_{2C} receptors are inhibitory (Jordan, 2005). In neurons of the nucleus tractus solitarius receiving vagal afferent input,

the selective 5-HT_{2B} receptor antagonist LY202715 significantly reduced the excitatory actions of BW723C86 on "intermediate" and "polysynaptic" cells (Sévoz-Couche et al., 2000).

Multiple, as opposed to single, applications of α-methyl-5-HT cause a long-lasting inhibition of both spontaneous and fictive inspiratory-related GABAergic neurotransmission to cardioinhibitory vagal neurons, which are prevented by the 5-HT_{2B} receptor antagonist SB204741 (Dergacheva et al., 2008). BW723C86 reversibly increases both the frequency and amplitude of miniature excitatory postsynaptic currents in cardiac vagal neurons. The facilitation evoked by α-methyl-5-HT is blocked by the 5-$HT_{2B/2C}$ receptor antagonist SB206553. Interestingly, the blockade of both NMDA and non-NMDA glutamatergic receptors did not prevent α-methyl-5-HT–evoked facilitation of miniature excitatory postsynaptic currents; however, the responses were blocked by P2 receptor antagonists (Dergacheva et al., 2008). These results indicate that activation of 5-HT_2 receptors facilitates excitatory purinergic, but not glutamatergic, neurotransmission to cardiac vagal neurons.

11. Amygdala and Anxiety. Animals administered the 5-HT_{2B} receptor agonist BW723C86 exhibit anxiolytic-like behavior in both the rat social interaction test and two conflict models of anxiety, the rat Geller-Seifter and marmoset conflict test (Kennett et al., 1995). Adult rat neurons in the medial amygdolid nucleus express 5-HT_{2B} receptors protein (Fig. 11), where local application of a 5-HT_{2B} receptor agonist displays anxiolytic activity in the social-interaction model but has little effect on behavior in a punished conflict model of anxiety (Kennett et al., 1996a,b). This 5-HT_{2B} receptor agonist also increased the time spent in feeding behavior of freely fed rats in observation cages over 15 minutes. The effect was also likely to be 5-HT_{2B} receptor–mediated, as no response to BW723C86 was evident in freely fed rats pretreated with the 5-$HT_{2C/2B}$ receptor antagonist SB206553. BW723C86 also reduced the frequency of grooming bouts of rats in observation cages (Kennett et al., 1997a). Finally, BW723C86 increased the number of punishments accepted in a rat Vogel drinking conflict paradigm over 3 minutes, as do anxiolytic benzodiazepine drugs. The antipunishment effect of BW723C86 was blocked by the 5-$HT_{2B/2C}$ receptor antagonists SB206553 or SB215505 but not by the selective 5-HT_{2C} receptor antagonist SB242084. Thus, the antipunishment action of BW723C86 is likely to be 5-HT_{2B} receptor–mediated (Kennett et al., 1998).

12. Sleep. 5-HT_{2A} and 5-HT_{2C} receptors modulate deep [slow wave (SW)] sleep and low-frequency EEG power in humans and rodents. Antagonists of 5-HT_{2A} and/or 5-HT_{2C} receptors have a well known slow-wave sleep–enhancing effect. In contrast, blockade of 5-HT_{2B} receptors increases motor activity and wakefulness along with decreased theta activity during wakefulness and REM sleep. The 5-HT_{2B} receptor antagonist SB215505 dose-dependently increases wakefulness at the expense of the intermediate stage of sleep, REM sleep, and slow-wave sleep and reduces low-frequency (<8 Hz) EEG power. In REM sleep, the 5-HT_{2B} receptor antagonist SB215505 dose-dependently decreases EEG power solely in the theta (6–9 Hz) band, primarily affecting the peak power value (7 Hz) (Kantor et al., 2004). 5-HT exerts a 5-HT_{2B} receptor–mediated facilitation of NREM sleep and an influence that is, respectively, inhibitory on NREM sleep and facilitatory on sleep apnea generation via 5-HT_{2A} receptors (Popa et al., 2005). $Htr_{2B}^{-/-}$ mice exhibited significantly increased wakefulness, with less NREM sleep, whereas REM sleep is not affected. Chronic oral intake of haloperidol restored the balance between wakefulness and NREM sleep in $Htr_{2B}^{-/-}$ mice (Pitychoutis et al., 2015). Activation of 5-HT_{2B} receptors may thus contribute to initiation of sleep and to theta generation during wakefulness and REM sleep under physiologic conditions. However, it should be kept in mind that, clinically, there have been no successes in insomnia/sleep disorders with 5-HT_2 receptor antagonists in spite of multiple attempts, at least with 5-HT_{2A} receptor antagonists.

J. Clinical Relevance

1. Feeding and Anorexigens. The hypophagic response to the anorexigen and 5-HT releaser, dexfenfluramine, observed in wild-type mice is absent in $Htr_{2B}^{-/-}$ mice or in wild-type mice treated with RS127445. The dexfenfluramine-induced hypothalamic peak of 5-HT release is strongly reduced in $Htr_{2B}^{-/-}$ awake mice

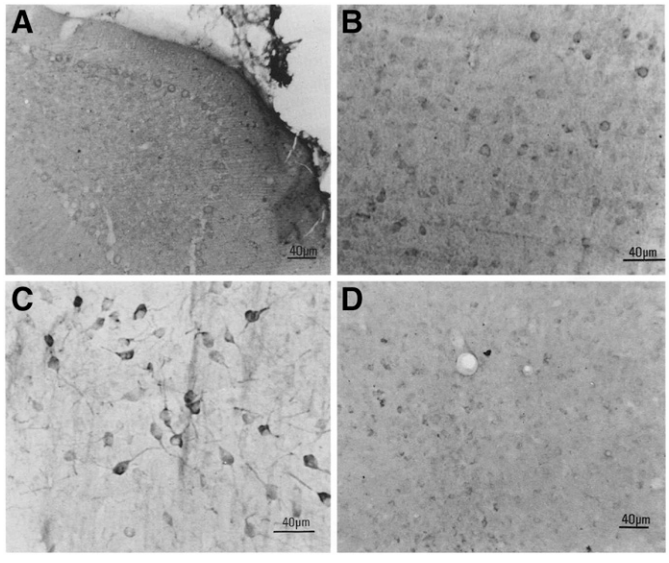

Fig. 11. 5-HT_{2B} receptor immunoreactivity in rat brain. Immunohistochemical detection of 5-HT_{2B} receptor immunoreactivity within Purkinje cells in the cerebellum (A), multipolar neurons in the lateral septum (B), multipolar and bipolar neurons in the medial amygdala (C), and cells in the dorsal hypothalamic nucleus (D). In each case, the staining was abolished in adjacent sections by coincubation with synthetic 5-HT_{2B} receptor peptide (data not shown). Adapted from Duxon et al. (1997) (with permission).

compared with control mice. Dexfenfluramine-induced 5-HT release is observed in synaptosomal preparation from wild-type mice but absent in $Htr_{2B}^{-/-}$ mice (Banas et al., 2011). 5-HT_{2B} receptor–induced NO production phosphorylates SERT and maximizes 5-HT uptake in raphe neuron primary culture. 5-HT_{2B} receptor–PKC coupling promotes additional phosphorylation of both SERT and Na^+, K^+-ATPase α-subunit, impairing the electrochemical gradient necessary for 5-HT uptake. Such 5-HT_{2B} receptor–mediated control of SERT activity operated in primary neurons from the raphe nuclei (Launay et al., 2006). Thus, activation of 5-HT_{2B} receptors is a limiting step in the SERT-dependent–releasing effect of dexfenfluramine, whereas other 5-HT receptors may act downstream with respect to feeding behavior.

2. Raphe and Antidepressant Activity. 5-HT neurotransmission is tightly regulated by autoreceptors that fine-tune 5-HT neurotransmission through negative feedback inhibition at the cell bodies (predominantly 5-HT_{1A}) or at the axon terminals (predominantly 5-HT_{1B}), although 5-HT_{2B} receptors may play different roles (McDevitt and Neumaier, 2011). The therapeutic effects induced by 5-HT–selective reuptake inhibitor (SSRI) antidepressants are initially triggered by blocking the SERT and rely on long-term adaptations of pre- and postsynaptic receptors. Long-term behavioral and neurogenic SSRI effects were abolished after either genetic or pharmacologic inactivation of 5-HT_{2B} receptors (Diaz et al., 2012). Conversely, direct agonist stimulation of 5-HT_{2B} receptors induced an SSRI-like response in behavioral and neurogenic assays. The 5-HT_{2B} receptor is expressed in raphe serotonergic neurons. The SSRI-induced increase in hippocampal extracellular 5-HT concentration is strongly reduced in the absence of functional 5-HT_{2B} receptors; hence, selective 5-HT_{2B} activation mimics SSRI effects (Diaz et al., 2012). Thus, the 5-HT_{2B} receptor modulates 5-HT neuron activity and may be required for the therapeutic actions of SSRIs.

3. Dopamine and Antidepressant Activity. Agomelatine, a potent melatonin MT1/MT2 receptor agonist, is an effective antidepressant and a potent 5-$HT_{2B/2C}$ receptor antagonist (Millan et al., 2003). Twice daily melatonin increases the number of spontaneously active dopamine neurons without affecting noradrenaline neurons. Long-term administration of either melatonin or the 5-HT_{2C} receptor antagonist SB242084 has no effect on the firing rate and burst parameters of 5-HT and dopamine neurons, whereas their combination enhances the number of spontaneously active dopamine neurons while leaving the firing of 5-HT neurons unchanged. The addition of the 5-HT_{2B} receptor antagonist LY266097 to the previous regimen, which by itself is devoid of effect, increases the activity of dopamine neurons (Chenu et al., 2014). Hence, the combination of melatonin receptor activation, 5-HT_{2C}, and 5-HT_{2B} receptor blockade results in a disinhibition of dopamine neurons, reproducing the antidepressant effect of agomelatine.

4. Serotonin (5-HT) Syndrome. The 5-HT syndrome occurs in humans after a combination of drugs inducing a massive increase in extracellular 5-HT or direct targeting of several 5-HT receptor subtypes [see Scotton et al. (2019)]. $Htr_{2B}^{-/-}$ mice are more prone to develop the 5-HT syndrome symptoms after administration of high dose of SSRI or the 5-HT precursor, 5-hydroxytryptophan, although increases in 5-HT plasma levels are similar in both genotypes (Diaz and Maroteaux, 2011).

5. Drug of Abuse. The "club drug" MDMA ("ecstasy") inhibits SERT activity, releasing 5-HT stores from nerve terminals. The subsequent activation of postsynaptic 5-HT receptors by released 5-HT is critical for the unique psychostimulatory effects of MDMA. Acute pharmacological inhibition or genetic ablation of the 5-HT_{2B} receptor in mice completely abolishes MDMA-induced hyperlocomotion and 5-HT release in nucleus accumbens and ventral tegmental area. The MDMA-stimulated release of endogenous 5-HT from superfused midbrain synaptosomes is 5-HT_{2B} receptor–dependent (Doly et al., 2008). $Htr_{2B}^{-/-}$ mice show no behavioral sensitization or conditioned place preference following MDMA. In addition, MDMA-induced reinstatement of conditioned place preference and locomotor sensitization are both abolished by RS127445 in mice, whereas MDMA-induced dopamine D1 receptor–dependent phosphorylation of extracellular regulated kinase in nucleus accumbens is abolished in mice lacking functional 5-HT_{2B} receptors. These results underpin the importance of 5-HT_{2B} receptors in the reinforcing properties of MDMA (Doly et al., 2009).

The selective 5-HT_{2B} receptor antagonist LY266097 reduces dopamine outflow in the ventral striatum/nucleus accumbens but not in the dorsal striatum (Auclair et al., 2010). The locomotor response of $Htr_{2B}^{-/-}$ mice to both dizocilpine and amphetamine is significantly enhanced compared with control mice (Pitychoutis et al., 2015). 5-HT_{2B} receptor antagonists reduce cocaine-induced hyperlocomotion independently of changes of subcortical dopamine outflow, supporting a regulatory control exerted by this receptor on ascending dopamine pathways (Devroye et al., 2015). Thus, 5-HT_{2B} receptors may also exert, in addition to 5-HT neurons, a facilitatory control on mesoaccumbens dopamine pathway activity.

6. Impulsivity. A functional stop codon in the human 5-HT_{2B} receptor gene enhances impulsive behavior (Bevilacqua et al., 2010). Especially under conditions in which control is impaired, the carriers of the stop codon are more vulnerable to alcohol and more impulsive upon drinking. Similarly, $Htr_{2B}^{-/-}$ mice display more impulsive choice in delayed discounting tasks, sought novelty, and are more active after receiving a D_1 dopamine receptor agonist (Bevilacqua et al., 2010). Interestingly, early onset schizophrenia is more prevalent in human

5-HT_{2B} receptor gene Q*20 carriers. Domains related to the positive, negative, and cognitive symptom clusters of schizophrenia are affected in $Htr_{2B}^{-/-}$ mice, with deficits in sensorimotor gating, selective attention, social interactions, learning, and memory processes. $Htr_{2B}^{-/-}$ mice show enhanced locomotor response to dizocilpine and amphetamine and alterations in sleep architecture. 5-HT_{2B} receptor ablation induces a region-selective decrease of dopamine and glutamate concentrations in the dorsal striatum. Importantly, selected schizophrenic-like phenotypes and endophenotypes are rescued by chronic haloperidol treatment (Pitychoutis et al., 2015). The phenotypes of $Htr_{2B}^{-/-}$ mice result from a combination of both the direct absence of 5-HT_{2B} receptor signaling and the resultant neural adaptations.

7. Fragile X Syndrome. Fragile X syndrome, caused by the loss of Fmr1 gene function, is the most common form of inherited mental retardation, with no current effective treatment. 5-HT_{2B} receptor activation moderately enhances Ras-PI3K/PKB signaling input, GluA1-dependent synaptic plasticity, and learning in Fmr1 knockout mice without causing anxiety-related side effects (Lim et al., 2014).

8. Cardiovascular Diseases. 5-HT_{2B} receptors are overexpressed in heart tissue from patients with congestive heart failure, in parallel to increased cytokine and norepinephrine plasma levels (Jaffre et al., 2009). 5-HT plasma levels are also increased in patients with heart failure and in rodents with cardiac hypertrophy induced by aortic constriction. Thus, 5-HT–induced cardiac hypertrophy and/or heart failure may be 5-HT_{2B} receptor–dependent or at least have responses likely exacerbated via the lack of 5-HT_{2B} receptor. In support of which, 5-HT_{2B} receptor KO mice do not develop isoproterenol-induced left-ventricular hypertrophy (Jaffré et al., 2004). Mice expressing the 5-HT_{2B} receptor exclusively in cardiomyocytes, similarly to global 5-HT_{2B} receptor–null mice, are resistant to isoproterenol-induced cardiac hypertrophy and dysfunction as well as to isoproterenol-induced increases in plasma cytokine levels (Jaffre et al., 2009). In primary culture of cardiac fibroblasts, angiotensin II– and isoproterenol-stimulated NOX activity is prevented by the selective 5-HT_{2B} receptor antagonist (SB215505). SB215505 prevents the increase in cardiac superoxide generation and hypertrophy in two models of cardiac hypertrophy (i.e., angiotensin II and isoproterenol infusions in mice) (Monassier et al., 2008). A functional interaction between AT_1 and 5-HT_{2B} receptors via a transinhibition mechanism may involve heterodimeric receptor complexes and trigger cytokine release in cardiac fibroblasts (Jaffre et al., 2009).

The 5-HT_{2B} receptor is involved in cardiac hypertrophy by acting directly on cardiac myocytes. After 2 weeks of aortic banding surgery, 5-HT_{2B} receptor mRNA and protein expression are increased. SB215505 significantly reduces the arising increase in heart weight, heart wall thickness, left-ventricular mass, and expression of the brain natriuretic peptide (BNP) but does not attenuate the upregulation of 5-HT_{2B} receptor protein expression in rats after aortic banding. Following in vitro mechanical stretch of cardiomyocytes and incubation with 5-HT, the level of 5-HT_{2B} receptors and BNP protein increases time-dependently. 5-HT_{2B} receptor siRNA applied to cardiomyocytes reversed both the increase of NF-κB translocation and BNP protein induced by 5-HT incubation plus mechanical stretch (Liang et al., 2006). 5-HT_{2B} receptors are involved in the generation of apoptotic events associated with cardiac remodeling during increased adrenergic stimulation (Bai et al., 2010). Thus, there is a dual role for 5-HT_{2B} receptors on both cardiomyocytes and cardiac fibroblasts in regulating cardiac hypertrophy in vivo.

9. Pulmonary Arterial Hypertension. Pulmonary arterial hypertension (PAH) is a progressive and often fatal disorder that results from increased pulmonary blood pressure associated with abnormal vascular proliferation. 5-HT is associated with the pathogenesis of PAH (Chan and Loscalzo, 2008). Therapeutics with PAH as a side effect (e.g., dexfenfluramine) are potent 5-HT releasers acting at SERT and/or 5-HT_{2B} receptor agonists (Weir et al., 2008). The blockade of 5-HT_{2B} receptors, either by genetic ($Htr_{2B}^{-/-}$) or pharmacologic inactivation (5-HT_{2B} receptor antagonist RS127445), completely prevents the development of hypoxia-induced pulmonary hypertension in mice and lung remodeling including the increase in vascular proliferation, elastase activity, and TGFβ1 levels (Launay et al., 2002). In the monocrotaline-induced pulmonary hypertension model, a number of 5-HT_{2B} receptor antagonists (terguride, PRX-08066, or C-122) reduce pulmonary pressure, arterial wall thickening, and lumen occlusion but maintained cardiac function (Porvasnik et al., 2010; Dumitrascu et al., 2011; Zopf et al., 2011). Pulmonary hypertension is associated with a substantial increase in 5-HT_{2B} receptor expression in the pulmonary arteries of both rodents and humans (Launay et al., 2002; Dumitrascu et al., 2011). Activation of 5-HT_{2B} receptors appears to be, therefore, a limiting step in the development of pulmonary hypertension. However, the restricted expression of 5-HT_{2B} receptors to bone marrow cells is necessary and sufficient for pulmonary hypertension to develop via an action at hematopoietic stem cell differentiation (Launay et al., 2012). There appears to be a limiting role of 5-HT_{2B} receptors in PAH development; thus, the contribution of 5-HT to PAH may be an extrapulmonary, and specifically hematopoietic, event.

10. Vascular Hypertension. Mesenteric arteries from DOCA-salt hypertensive rodents predominantly contract via 5-HT_{2B} receptors; they display an increase in 5-HT_{2B} receptor mRNA and receptors (Watts et al., 1996). The isolated endothelium-denuded superior mesenteric artery of DOCA-salt rats displays a marked

increase in the maximal contraction to the 5-HT_{2B} receptor agonist BW723C86. In chronically instrumented rats, the 5-HT_{2B} receptor antagonist LY272015 significantly reduces mean blood pressure (Watts and Fink, 1999). LY272015 also inhibited 5-HT–induced contractions in aorta from rats made hypertensive by exposure to the nitric-oxide synthase inhibitor N(omega)-nitro-L-arginine, whereas ketanserin was inactive (Russell et al., 2002). Thus, the 5-HT_{2B} receptor appears to play an important role in 5-HT–induced contraction in hypertensive rodent arteries.

11. Fibrosis. 5-HT increases proliferation and collagen synthesis of lung fibroblasts. 5-HT concentrations in lung homogenates increase significantly following bleomycin-induced fibrosis, paralleled with an increased expression of 5-HT_{2A} and 5-HT_{2B} receptors (Königshoff et al., 2010). Blockade of 5-HT_{2B} receptors by SB215505 reduces bleomycin-induced lung fibrosis, with reduced lung collagen levels and procollagen 1 and 3 mRNA expression. 5-HT_{2B} receptor antagonists decrease levels of lung TGFβ1 mRNA, connective tissue growth factor and plasminogen activator inhibitor-1, and JunD mRNA, consistent with their antifibrotic activity. Interestingly, the 5-HT_{2B} receptor is strongly overexpressed in fibroblastic foci in human idiopathic pulmonary fibrosis (Fabre et al., 2008). Thus, it is likely that 5-HT–induced lung fibrosis is controlled by 5-HT_{2B} receptors regulating TGFβ1 levels.

In the liver, fibrogenic HSC, which are negative regulators of hepatocyte regeneration, are known to express both 5-HT_{2A} and 5-HT_{2B} receptors, which may regulate TGFβ1 and Smad signaling (Li et al., 2006). HSCs play a key role in hepatic wound healing and fibrosis. After HSC activation, expression of 5-HT_{2A} and 5-HT_{2B} receptors is around 100- and 50-fold that of quiescent cells, respectively. The 5-HT_{2B} receptor expression is strongly associated with fibrotic tissue in diseased liver. 5-HT_2 receptor antagonist–treated HSCs display suppressed proliferation and increased apoptosis. 5-HT synergizes with platelet-derived growth factor to stimulate HSC proliferation (Ruddell et al., 2006). In contrast to quiescent cells, activated HSCs exhibit [Ca^{2+}]i transients following treatment with 5-HT, which is inhibited by ritanserin. Expression of type 1 inositol-5′-triphosphate receptor and type 2 sarcoplasmic/endoplasmic reticulum Ca^{2+} ATPase is also increased during activation of HSCs and serves as the major isotype for ER Ca^{2+} storage and release in activated HSCs. ER Ca^{2+}–binding chaperone proteins, including calreticulin, calnexin, and calsequestrin, are upregulated following activation of HSCs (Park et al., 2011). 5-HT_{2B} receptor stimulation of HSCs increases the expression of TGFβ1 (a powerful suppressor of hepatocyte proliferation) via ERK/JunD signaling. Similar effects are evident in mice lacking 5-HT_{2B} receptor or JunD or when HSCs have been selectively depleted. 5-HT_{2B} receptor blockade attenuates fibrogenesis and improves liver function in disease models in which fibrosis is pre-established and progressive (Ebrahimkhani et al., 2011). Therefore, the hepatic 5-HT_{2B} receptor appears to have a dual role, promoting regeneration in physiologic conditions and fibrosis in pathologic conditions.

Dermal fibrosis is reduced in $Htr_{2B}^{-/-}$ mice using both inducible and genetic models of fibrosis. Pharmacologic inactivation of the 5-HT_{2B} receptor effectively prevents the onset of experimental fibrosis and ameliorates established fibrosis by decreasing mRNA levels of TGFβ1, connective growth factor, plasminogen activator inhibitor-1, and Smad-3 (Dees et al., 2011). Moreover, inhibition of platelet activation prevents fibrosis in models of skin fibrosis. In TPH1-deficient mice, the rate-limiting enzyme for 5-HT production outside the central nervous system shows reduced experimental skin fibrosis (Dees et al., 2011). Skin fibrosis is thus controlled by 5-HT_{2B} receptors via regulation of TGFβ1 levels.

Treatment of neonatal rat cardiac fibroblasts with 5-HT increases the expression of smooth muscle α-actin, a marker of fibroblast differentiation into myofibroblasts and stimulated cardiac fibroblast migration. 5-HT enhances secretion of TGFβ1 and expression of MMPs in cardiac fibroblasts (Yabanoglu et al., 2009). 5-HT– or AngII-stimulated cytokine release and secretion of TGFβ1 in adult cardiac fibroblasts is sensitive to 5-HT_{2B} receptor blockade. Treatments with epidermal growth factor receptor (ErbB1/4)-selective inhibitors or with selective inhibitors of MMPs also abolished AngII- and 5-HT–induced cytokine release. Finally, the use of $HB\text{-}EGF^{-/-}$ cardiac fibroblasts confirmed that epidermal growth factor receptor stimulation is absolutely required for AngII- and 5-HT–dependent cytokine release (Jaffre et al., 2009). Collectively, these results highlight that a convergent action of norepinephrine, AngII, and 5-HT via interactions between AT_1 and 5-HT_{2B} receptors coexpressed by noncardiomyocytes is limiting key events in cardiac hypertrophy.

12. Valvular Heart Disease. Valvular heart disease occurs in over 65% of patients with carcinoid syndrome and is characterized by fibrous thickening of cardiac valves, leading to heart failure [for review, see Roth (2007)]. High plasma 5-HT levels correlate with valvular abnormalities detected by cardiac catheterization and echocardiography (Robiolio et al., 1995). The similarity of lesions in carcinoid heart disease and in methysergide-associated valvular disease suggested a direct stimulation of myofibroblast growth by an unknown 5-HT receptor agonism (Hendrikx et al., 1996). The increase in fenfluramine-associated valvular heart disease raised concerns that other 5-HT–relevant medications might also increase the risk of valvular heart disease (Connolly et al., 1997). Dexfenfluramine had been approved in the United States for long-term use as an appetite suppressant until it was associated with valvular heart disease. The valvular changes

(myofibroblast proliferation) are histopathologically indistinguishable from those observed in carcinoid disease or following long-term exposure to 5-HT_2 receptor–preferring ergot drugs (ergotamine, methysergide). The amphetamine derivative, MDMA ("ecstasy"), and its N-demethylated metabolite, 3,4-methylenedioxyamphetamine (MDA), both preferentially bind to and activate human recombinant 5-HT_{2B} receptors. Like fenfluramine and norfenfluramine, they elicit mitogenic responses in human valvular interstitial cells via likely activation of 5-HT_{2B} receptors (Setola et al., 2003). Based on these strikingly similar echocardiographic and histopathologic features, it is now considered that ergot-derived dopamine agonists (e.g., pergolide and cabergoline) cause a valvular heart disease nearly identical to that seen in patients with carcinoid syndrome (Horvath et al., 2004). Population studies of patients with Parkinson disease compared with nonparkinsonian controls show that pergolide and cabergoline have a similar risk of inducing fibrotic changes in cardiac valve leaflets. Pergolide and cabergoline have high affinity for the 5-HT_{2B} receptors. The frequency of moderate-to-severe regurgitation in at least one heart valve was higher in patients receiving cabergoline or pergolide compared with patients taking nonergot agonists or controls, and the incidence of new-onset valvulopathy was relatively high in patients taking the ergot-derived drugs (Antonini and Poewe, 2007; Roth, 2007). A simultaneous mitral bioprosthesis hypertrophic scaring and native aortic valve fibrosis was recently reported following benfluorex therapy. The bioprosthesis and aortic valves exhibit similar histopathological lesions. Thickening and plaque deposits made by smooth muscle α-actin– and vimentin-positive cells in a glycosaminoglycan matrix were observed, supporting that activation of the 5-HT_{2B} receptor by norfenfluramine may trigger the development of drug-induced heart disease (Ayme-Dietrich et al., 2012).

5-HT_{2B} and 5-HT_{2A} receptor transcripts are reported to be present in heart valves, whereas no 5-HT_{2C} receptor transcript is detectable. Preferential stimulation of valvular 5-HT_{2B} receptors (with or without accompanying 5-HT_{2A} receptor activation) may contribute to valvular fibroplasia in humans (Fitzgerald et al., 2000). Mitral valve regurgitation has been associated with increased mRNA expression of valvular 5-HT_{2B} receptors and SERT in pigs (Cremer et al., 2015b). Canine myxomatous mitral valve disease was associated with higher expression of 5-HT_{2B} receptors in mitral valve (Cremer et al., 2015a).

These findings suggest that 5-HT_{2B} receptor signaling links vascular damage and platelet activation to tissue remodeling and identify the 5-HT_{2B} receptor as a novel potential therapeutic target to treat valvular heart diseases. As a result of these investigations, the development of 5-HT_{2B} receptor agonists have been banned by the FDA.

13. Acute Pain. 5-HT released from mast cells or platelets in peripheral tissues is an important inflammatory mediator of pain. The involvement of 5-HT in pain is complex since it can inhibit or facilitate nociceptive transmission because of the presence of multiple functionally opposing 5-HT receptors on peripheral and central nociceptors. An acute injection of 5-HT or the 5-HT_2 receptor, agonist α-methyl-5-HT, into hindpaws of mice induces significant hyperalgesia to mechanical stimuli, which can be inhibited by the 5-$HT_{2B/2C}$ receptor antagonist SB206553, which also blocks 5-HT–induced transient [Ca^{2+}] signaling in DRG neurons. 5-HT_{2B} receptors are involved in mechanical hyperalgesia in mice (Lin et al., 2011). 5-HT released from descending pain modulation pathways to the dorsal horn is crucial in spinal nociception processing. Local peripheral ipsilateral, but not contralateral, administration of RS127445 significantly prevents formalin-induced flinching behavior. Moreover, local peripheral ipsilateral, but not contralateral, administration of the 5-HT_2 receptor agonist DOI augmented formalin-induced nociception. The local pronociceptive effect of DOI is prevented by local RS127445. Moreover, intrathecal RS127445 also prevented formalin-induced nociception. By contrast, spinal DOI increases flinching behavior induced by formalin. The spinal pronociceptive effect of DOI is prevented by intrathecal RS127445 (Cervantes-Durán et al., 2012). It is therefore evident that 5-HT_{2B} receptors play a pronociceptive role in peripheral as well as spinal sites in the rat formalin test.

14. Chronic Pain. In rats subjected to spinal nerve ligation, the 5-HT_{2B} receptor agonist BW723C86 enhances evoked potentials, whereas the 5-HT_{2B} receptor antagonist SB204741 depresses them. Spinal hyperexcitation promoted by 5-HT_{2B} receptors has been proposed as a pathogenic pathway contributing to pain (Aira et al., 2010). Plasticity of spinal serotonergic neurotransmission selectively reduces spinal μ-opioid receptor mechanisms via increased expression of 5-HT_{2B} receptors in dorsal horn neurons also expressing the μ-opioid receptor (Aira et al., 2012). The involvement of glutamate receptors in dorsal neuron hyperexcitation can also be promoted by 5-HT_{2B} receptor after spinal nerve ligation. Augmentation of C-fiber–evoked potentials by spinal superfusion with the 5-HT_{2B} receptor agonist BW723C86 in nerve-ligated rats is impeded by coadministration of NMDA receptor antagonist. Evoked potentials are increased by NMDA receptor agonist in nerve-injured rats, irrespective of simultaneous 5-HT_{2B} receptor blockade by SB204741. In uninjured rats, NMDA receptor agonist enhances evoked potentials in the presence of BW723C86 but not if administered alone. Blockade of 5-HT_{2B} receptors with the selective antagonist SB204741 after spinal nerve ligation bilaterally decreases phosphorylation of the NMDA receptor subunit NR1 (pNR1), in the synaptic fraction, and colocalization of both PKCγ and pNR1 with

postsynaptic marker PSD-95. Delivery of SB204741 bilaterally attenuates thermal and mechanical allodynia occurring after spinal nerve ligation, at early time periods, day 2 postinjury. The transient activation of the PKCγ/NMDA receptor pathway is critically involved in 5-HT_{2B} receptor–mediated facilitation in the spinal nerve ligation model (Aira et al., 2013). The adaptor protein NADH dehydrogenase subunit 2 (ND2) is involved in NR1 phosphorylation and spinal hyperexcitability secondary to peripheral nerve injury. Spinal nerve ligation is followed by increased colocalization of ND2 with pNR1. C-fiber–evoked dorsal horn field potentials are increased after spinal nerve ligation by superfusion with an NMDA receptor agonist. This increased postsynaptic upregulation of ND2/pNR1 can be prevented by prior administration of selective 5-HT_{2B} antagonist SB204741 (Aira et al., 2014). Thus, NMDA receptor phosphorylation is instrumental in coupling 5-HT_{2B} receptor–mediated input to NMDA receptor expressing synapses in spinal hyperexcitation involved in pain.

15. Neuropathic Pain. 5-HT was also implicated in a rat model of neuropathic pain evoked by chronic constriction injury (CCI) of the sciatic nerve. 5-HT_{2B} receptor activation has been reported to both prevent and reduce CCI-induced allodynia at 3 weeks postinjury. Intrathecal administration of the 5-HT_{2B} receptor agonist BW723C86 attenuated established mechanical and cold allodynia, an effect prevented by coinjection of RS127445. A single application of BW723C86 on the sciatic nerve concomitantly to CCI dose-dependently prevented mechanical allodynia and reduced cold allodynia 17 days after CCI. This behavioral effect is accompanied with a marked decrease in macrophage infiltration into the sciatic nerve and, in the DRG, with an attenuated abnormal expression of several markers associated with local neuroinflammation and neuropathic pain. CCI results in a marked upregulation of 5-HT_{2B} receptor expression in sciatic nerve and DRG. In the latter structure, it is biphasic, consisting of a transient early increase 2 days after surgery (around 23-fold) before neuropathic pain emergence, followed by a steady (around fivefold) increase that remains relatively constant until the pain disappeared. In DRG and sciatic nerve, 5-HT_{2B} receptors are immunolocalized on sensory neurons and infiltrating macrophages (Urtikova et al., 2012).

It thus appears that 5-HT_{2B} receptor involvement in pain takes place at various sites and time periods; early events are more pronociceptive, whereas at later stages, this receptor contribution may be more antinociceptive, although this varies according to the animal models.

16. Spasticity in Amyotrophic Lateral Sclerosis. Spinal cord injury leads to an initial phase of hyporeflexia followed by hyperreflexia, often referred to as spasticity. Spasticity is a common and disabling symptom also observed in patients with amyotrophic lateral sclerosis, a disease that can affect both upper and lower motor neurons. A rat tail spasticity model with a caudal spinal transection demonstrates 5-HT_{2B} receptor downregulation at 21 days postinjury (Wienecke et al., 2010). Motoneurons, which recover from denervation, function autonomously, exhibiting large persistent calcium currents that help with functional recovery and contribute to uncontrolled muscle spasms. Application of agonists relatively selective to 5-HT_{2B} receptors (including BW723C86) increase persistent calcium currents. 5-HT_{2B} receptors on motoneurons ultimately contribute to recovery of motoneuron function and emergence of spasms (Murray et al., 2011). In amyotrophic lateral sclerosis, spasticity is traditionally thought to be the result of degeneration of the upper motor neurons in the cerebral cortex, although degeneration of other neuronal types, particularly 5-HT neurons, might also underlie the spasticity. In superoxide dismutase 1 (G86R) mice, a transgenic model of amyotrophic lateral sclerosis, 5-HT levels are decreased in brainstem and spinal cord before onset of motor symptoms. Furthermore, there is noticeable atrophy of 5-HT neuronal cell bodies along with neuritic degeneration at disease onset. In superoxide dismutase 1 (G86R) mice, tail muscle spastic-like contractions occur at end-stage. Importantly, they are abolished by the 5-$HT_{2B/2C}$ receptors inverse agonist, SB206553. In keeping with this, 5-HT_{2B} receptor expression is strongly increased at disease onset (Dentel et al., 2013). In summary, 5-HT_{2B} receptors on motoneurons can become constitutively active after injury and ultimately contribute to some recovery of motoneuron function and emergence of spasms.

17. Migraine. A role for 5-HT in migraine is supported by changes in circulating levels of 5-HT and its metabolites during the phases of a migraine attack. A migraine headache is thought to be transmitted by the trigeminal nerve from the meninges and their associated blood vessels. Correlation of the receptor affinities with the potencies of drugs used in migraine prophylaxis demonstrates correlations with the 5-HT_{2B} receptor, and various human meningeal tissues express 5-HT_{2B} mRNAs (Schmuck et al., 1996). The 5-HT_{2B} receptor can activate the release of the smooth muscle relaxant NO and induce relaxation of the cerebral arteries and the jugular vein. 5-HT_{2B} receptors expressed by endothelial cells of meningeal blood vessels may trigger migraine headache through the formation of NO, which results in the dilation of cerebral blood vessels and the concomitant activation of sensory trigeminovascular afferents, thus initiating the manifestation of head pain (Johnson et al., 2003). In addition, a genetic study identified 5-HT_{2B} receptors as a susceptibility gene to migraine (Corominas et al., 2010). Endothelial 5-HT_{2B} receptors may thus trigger dilation of meningeal blood vessels, which by activating sensory trigeminovascular afferents, induces head pain.

18. Visceral Pain. 5-HT_{2B} receptors are involved in signaling from the colon in rats, in which there is visceral hypersensitivity. Oral administration of RS127445 inhibits visceral hypersensitivity provoked by restraint stress without significant effect on the visceral nociceptive threshold of naive rats (Ohashi-Doi et al., 2010). Moreover, when administered intracerebroventricularly, RS127445 also decreases the number of pain behaviors during noxious colorectal distension. A selective 5-HT_{2B} receptor antagonist has thus been proposed to have therapeutic potential for the treatment of gut disorders characterized by visceral hypersensitivity (O'Mahony et al., 2010). The 5-HT_{2B} receptor appears thus to be involved in regulating sensory pathways but only under hyperalgesic conditions, suggesting the possible utility of 5-HT_{2B} receptor antagonism in reducing visceral hypersensitivity in patients with irritable bowel syndrome and other hypersensitivity conditions.

19. Bones. 5-HT_{2B} receptor mRNA, which is undetectable in anaplastic osteoblasts, appears in differentiated and matured osteoblasts (Bliziotes et al., 2001; Westbroek et al., 2001). The differentiation and maturation of osteoblasts might thus be regulated by 5-HT_{2B} receptor activation (Hirai et al., 2009). Of interest, $Htr_{2B}^{-/-}$ female mice display reduced bone density from the age of 4 months that intensifies by 12 and 18 months. This histomorphometrically confirms that osteopenia is due to reduced bone formation (Collet et al., 2008). Using the osteoprogenitor cell line C1, blockade of 5-HT_{2B} receptor intrinsic activity affects the efficiency of mineralization by decreasing calcium incorporation. Optimal bone matrix mineralization involves both NO and PLA2 signaling pathways, and the 5-HT_{2B} receptor promotes prostaglandin E2 production through cyclooxygenase activation. When C1 osteoblasts undergo conversion into osteocyte-like cells, cyclooxygenase activity is quenched. The 5-HT_{2B} receptor contributes in an autocrine manner to osteogenic differentiation (Locker et al., 2006). There is a functional link between the 5-HT_{2B} receptor and the activity of the tissue nonspecific alkaline phosphatase (TNAP). Agonist stimulation of the receptor increases TNAP activity during the initial mineralization phase, whereas inhibition of 5-HT_{2B} receptor intrinsic activity prevents TNAP activation. In contrast, agonist stimulation of the receptor further increased TNAP activity during the initial mineralization phase. The 5-HT_{2B} receptor couples to PLA2 pathway and prostaglandin production at the beginning of mineral deposition. The 5-HT_{2B} receptor also controls leukotriene synthesis via PLA2 at the terminal stages of differentiation. These two 5-HT_{2B} receptor–dependent eicosanoid productions delineate distinct time windows of TNAP regulation during the osteogenic program. Finally, prostaglandins or leukotrienes relay the post-translational activation of TNAP via stimulation of the phosphatidylinositol-specific phospholipase C. In agreement with this, primary calvarial osteoblasts from $Htr_{2B}^{-/-}$ mice exhibit defects in TNAP activity (Baudry et al., 2010). Brain 5-HT may indirectly favor bone mass accrual following activation of 5-HT_{2C} receptors on ventromedial hypothalamic neurons and 5-HT_{2B} receptors on arcuate neurons (Yadav et al., 2009). Compared with control osteoblasts, the lack of 5-HT_{2B} receptors is associated with a 10-fold overproduction of prostacyclin, and the specific prostacyclin synthase inhibitor (U51605) totally rescues osteoblast aggregation and matrix mineralization in $Htr_{2B}^{-/-}$ osteoblasts without effect on WT osteoblasts. Prostacyclin is the endogenous ligand of PPAR-β/δ, and its inhibition in $Htr_{2B}^{-/-}$ cells totally rescues the alkaline phosphatase and osteopontin mRNA levels, cell-cell adhesion, and matrix mineralization. The absence of 5-HT_{2B} receptors leads to the overproduction of prostacyclin, inducing reduced osteoblast differentiation because of PPAR-β/δ –dependent target regulation and defective cell-cell adhesion and matrix mineralization (Chabbi-Achengli et al., 2013). Of relevance, the 5-HT_{2A} receptor is expressed only in osteoblasts, whereas 5-HT_{2B} receptor expression increases from precursor to mature osteoclasts (Hodge et al., 2013b). The 5-HT_{2B} receptor therefore appears to contribute in an autocrine manner to osteogenic differentiation.

20. Teeth Development. Periodontal diseases occur in patients treated with antidepressants such as SSRIs (e.g., fluoxetine). In the molar teeth of $Htr_{2B}^{-/-}$ mice, rod curvatures and twisting are altered compared with WT mice, suggesting involvement of the 5-HT_{2B} receptor at early stages of enamel formation. The volume of the enamel layer in $Htr_{2B}^{-/-}$ mice is also reduced, with smaller crystallite thickness. The outer aprismatic enamel border is 1.5- to twofold larger in $Htr_{2B}^{-/-}$ compared with WT mice. Finally, although no noticeable difference is observed in dentin, the micro-CT three-dimensional pulp reconstruction reveals a decrease in both length and width of dentin formation in the root canals of the $Htr_{2B}^{-/-}$ mice (Dimitrova-Nakov et al., 2014). Therefore, 5-HT_{2B} receptors may mediate some harmful effects of long-term use of SSRIs on bone and teeth regeneration.

21. Cancers.

a. Carcinoid tumors. 5-HT_{2B} receptor expression is observed in spontaneous human carcinoid tumors, along with coupling to $p21^{ras}$ activation (Launay et al., 1996). The tumor proliferative activity of small intestinal neuroendocrine tumors (including cell growth and the development of desmoplasia) is associated with the particular microenvironment in the peritoneum, and tumor cells support this necessary milieu through the secretion of profibrotic/angiogenetic factors (Svejda et al., 2010).

b. Breast tumors. Increased 5-HT biosynthetic capacity accompanied by multiple changes in 5-HT receptor expression and signaling favor malignant progression of

human breast cancer cells. Among them, expression of 5-HT_{2B} receptors is increased (Pai et al., 2009). 5-HT_{2B} receptor mRNA expression is lower in basal estrogen receptor–negative tumors compared with luminal tumors, which are most commonly estrogen receptor–positive. 5-HT_{2B} receptor mRNA is elevated in carcinomas, increased with tumor stage, and higher in lymph node–positive tumors compared with node-negative tumors. c-Myc transformation induces an increase in 5-HT_{2B} receptor expression (Pai et al., 2009). In human breast cancer, there is a significant correlation of 5-HT_{2B} receptor expression with estrogen receptor-α expression (Kopparapu et al., 2013).

c. Melanoma. Uveal (ocular) melanoma is an aggressive cancer that often forms undetectable micrometastases before diagnosis of the primary tumor. High increases in 5-HT_{2B} receptor mRNA are evident in all uveal melanomas with monosomy 3 compared with low expression in all tumors with disomy 3. As monosomy 3 is associated with metastatic disease, 5-HT_{2B} receptor expression has been proposed as a marker to identify patients with poor prognosis (Tschentscher et al., 2003). The 5-HT_{2B} receptor gene is among the genes showing the highest overexpression in class 2 uveal melanoma (van Gils et al., 2008). A PCR-based 15-gene assay comprising 12 discriminating genes, including the 5-HT_{2B} receptor gene, is now part of a prognostic assay, which provides an important addition to help manage patients with uveal melanoma (Onken et al., 2010) by distinguishing whether uveal melanomas contain liver metastases and thus aid in the diagnosis and prevention of uveal melanoma liver metastases based on their different features (Zhang et al., 2014a).

d. Prostate cancer. Prostate cancer is the most commonly diagnosed noncutaneous cancer in men. Despite this fact, many of the genetic changes that coincide with prostate cancer progression remain enigmatic. The 5-HT_{2B} receptor has been shown to be upregulated in tumors (Magee et al., 2001). Overexpression of receptors to neuroendocrine cell products may contribute to development of hormone-refractory prostate cancer. Immunostaining for the 5-HT_{2B} receptor is evident in low-grade and high-grade tumors, prostatic intraepithelial neoplastic and benign prostatic hyperplasia cells, and vascular endothelial cells. Antagonists for the 5-HT_{2B} receptor inhibit proliferation of prostate cancer cells in a dose-dependent manner (Dizeyi et al., 2005).

e. Adrenocortical carcinoma. Gene expression profiles of adrenocortical tumors demonstrate underexpression of 5-HT_{2B} receptor mRNA as a marker of malignant adrenocortical carcinoma (Fernandez-Ranvier et al., 2008). Analysis of biomarkers of malignancy of adrenocortical cancers in a meta-analysis suggests the combination of overexpressed anillin and underexpressed 5-HT_{2B} receptor mRNA to be the best predictor of malignancy (Zsippai et al., 2011). However, in adrenocorticotropin-dependent adrenal hyperplasia, the mechanisms responsible for the ectopic adrenal expression of glucose-dependent insulinotropic peptide (GDIP) receptor in GDIP-dependent Cushing's syndrome are unknown. Chronic adrenal stimulation by GDIP in GDIP-dependent adrenocorticotropic hormone–independent macronodular adrenal hyperplasia leads to the significant induction of genes for the GPR54, 5-HT_{2B}, GPR4, and endothelial differentiation sphingolipid EDG8 receptor (Lampron et al., 2006).

f. Hepatocellular carcinoma. Among 64 genes for which mRNA expression differed between non–hepatitis B and non–hepatitis C compared with hepatitis C–type hepatocellular carcinoma (HCC), the most affected is the gene for the 5-HT_{2B} receptor (Iizuka et al., 2004). The function of 5-HT as a survival factor of HCC cells has been demonstrated; activation of the 5-HT_{2B} receptor leads to sustained phosphorylation of two downstream targets of mTOR, p70S6K and 4E-BP1, thereby facilitating survival and inhibiting autophagy. Inhibiting the 5-HT_{2B} receptor reduces cancer cell growth in vitro and in vivo. The presence of 5-HT_{2B} receptors in HCC and the activation of autophagy-related mechanisms provide novel insights of 5-HT in cancer biology and propose 5-HT–mediated signaling as a therapeutic target (Soll et al., 2010). 5-HT_{1B} and 5-HT_{2B} receptors are expressed, respectively, in around 32% and 35% of the patients with hepatocellular cancer. Both receptors are associated with an increased proliferation index (Soll et al., 2012). The 5-HT_{2B} receptor mediates 5-HT–induced proliferation in the serum-deprived HCC Huh7 cells. Additionally, selective 5-HT_{2B} receptor antagonism using SB204741 in Huh7 cells decreases the expression of FOXO3a (Liang et al., 2013).

g. T-cell leukemia. A proteasome inhibitor, bortezomib, is a potential therapeutic agent to treat adult T-cell leukemia. A network including the 5-HT_{2B} receptor was identified that converges upon the secreted protein acidic and rich in cysteine gene, a tumor-invasiveness–related gene, which may act as a modulator of bortezomib-induced cell death in adult T-cell leukemia cells (Ohyashiki et al., 2008).

h. Myosarcoma. In the pathogenesis of uterine leiomyosarcoma, there is a fourfold overexpression of the 5-HT_{2B} receptor gene, and it is one of the most overexpressed genes (Arslan et al., 2005; Matsumura et al., 2006).

i. Tumor angiogenesis. 5-HT does not enhance colon cancer tumor cell proliferation but may act as a regulator of angiogenesis by reducing the expression of MMP-12 and lower levels of angiostatin—an endogenous inhibitor of angiogenesis (Nocito et al., 2008). 5-HT stimulates the phosphorylation of ERK1/2 in bovine endothelial cells, and the 5-HT_{2B} receptor plays a role in the activation of eNOS in human endothelial cells. In SB204741-treated mice, the selective blockade of the 5-HT_{2B} receptor results in the reduction of tumor angiogenesis and growth through the inhibition effect of ERK1/2 and eNOS (Asada et al., 2009). Therefore, the 5-HT_{2B} receptor may participate in tumor angiogenesis.

22. Clinically Relevant Knowledge Gained from the Gene.

a. The human 5-HT$_{2B}$ receptor gene. The human 5-HT$_{2B}$ receptor gene (MIM 601122) is located on chromosome 2q37.1 (Le Coniat et al., 1996). The human 5-HT$_{2B}$ receptor gene rate of evolution displays high conservation in primates (Andrés et al., 2007). Within the 23 sites that are highly conserved among primates and that have changed on the modern human lineage after separation from Denisovan ancestors, one (D216N) is found in ECL2 of the 5-HT$_{2B}$ receptor, suggesting that crucial aspects of synaptic transmission involving the receptor may have changed in modern humans (Meyer et al., 2012). There is evidence of the contribution of 5-HT$_{2B}$ receptor gene variants to intelligence quotation, intellectual disability, and language onset delay in patients with autism spectrum disorders (ASD) (Hervas et al., 2014).

The 5-HT$_{2B}$ receptor gene was identified as a susceptibility gene for impulsivity disorders; one single-nucleotide polymorphism (SNP) introducing a stop codon after amino acid 21 was found more frequently in severely impulsive individuals presenting suicidal behavior (Bevilacqua et al., 2010). In the same cohort, early-onset schizophrenia was more prevalent in 5-HT$_{2B}$ receptor gene Q*20 carriers. Other work testing SNPs within the 5-HT$_{2B}$ receptor gene for potential associations with the behavioral inhibition system and the three components of the behavioral approach system (fun-seeking, drive, and reward responsiveness) in a Han Chinese sample found four 5-HT$_{2B}$ receptor gene SNPs significantly associated with behavioral approach system fun-seeking (Zhu et al., 2012a).

Several SNPs confer a double-mutant R6G/E42G of the receptor protein associated with drug abuse, suggesting that 5-HT$_{2B}$ receptor contributes to pathways that are involved in drug dependence (Lin et al., 2004). Peripheral blood DNA methylation levels of CpGs in the promoter regions were examined in African Americans and European Americans. In European Americans, six CpGs in the 5-HT$_{2B}$ receptor gene promoter are significantly hypermethylated in alcohol-dependent cases (Zhang et al., 2013a).

An association study on susceptibility to migraine in a Spanish population supports the involvement of the 5-HT$_{2B}$ receptor gene and the monoamine oxidase A gene in the genetic predisposition to migraine without aura (Corominas et al., 2010). Finally, by investigating the 5-HT$_{2B}$ receptor gene in patients who developed pulmonary hypertension after use of fenfluramine, a heterozygous mutation was identified in one female patient who, 5 years earlier, had followed a 9-month anorexigen treatment (Blanpain et al., 2003). This heterozygous mutation R393X in the 5-HT$_{2B}$ receptor generates a carboxy terminus-truncated receptor characterized by a switch of coupling from Gαq/11 to Gα13, reduced NOS activation, and an increase in cell proliferation, modifications relevant to pathophysiological vasoconstriction (Deraet et al., 2005).

IX. 5-HT$_{2C}$ Receptor

A. Introduction

The 5-HT$_{2C}$ receptor is a G protein–coupled receptor (GPCR) with the characteristic seven-transmembrane domain structure with an extracellular N terminus and intracellular C terminus. Binding of 5-HT to the 5-HT$_{2C}$ receptor results in a conformational change that catalyzes the diffusion of multiple second messenger effectors. The canonical G protein–dependent signaling through the 5-HT$_{2C}$ receptor is engendered by 5-HT–stimulated coupling predominantly to G$\alpha_{q/11}$ to activate the enzyme phospholipase C (PLC), which generates phosphoinositide hydrolysis and intracellular calcium (Ca_i^{2+}) mobilization (Conn and Sanders-Bush, 1986a; Hoyer et al., 1989c; Chang et al., 2000).The 5-HT$_{2C}$ receptor also signals through other second messengers, which can include phospholipase D (PLD) and phospholipase A_2 (PLA_2), cyclic nucleotides, and extracellular signal–regulated kinases (Berg et al., 1994, 1998b; Kaufman et al., 1995; Werry et al., 2005). Landmarks in the progress of 5-HT$_{2C}$ receptor research are illustrated in Fig. 12 [see also Palacios et al. (2017)].

The gene for the human 5-HT$_{2C}$ receptor (*HTR2C*) was cloned and localized to the X chromosome (Xq24) in the early 1990s (Yu et al., 1991; Milatovich et al., 1992; Stam et al., 1994; Xie et al., 1996). The genomic DNA for the 5-HT$_{2C}$ receptor is divided into six exons with five introns (Xie et al., 1996), whereas the coding region contains four exons with three introns (Stam et al., 1994). The gene encodes a 458-amino-acid protein in humans and 460-amino-acid protein in rats, which share 90% amino acid homology (Saltzman et al., 1991). The 5-HT$_{2C}$ receptor is the only GPCR known to undergo RNA editing, resulting in the functional expression of multiple isoforms of the receptor that differ in their distribution, pharmacology, and signaling capabilities.

The 5-HT$_{2C}$ receptor was first identified in choroid plexus tissue by receptor autoradiography as a highly expressed binding site with high affinity for radioligands that bind to the 5-HT$_1$ receptor family (Pazos et al., 1984; Yagaloff and Hartig, 1985). This binding site displayed a different pharmacological profile from the known 5-HT$_{1A}$ receptor and 5-HT$_{1B}$ receptor and thus was originally named 5-HT$_{1C}$ receptor. It was cloned by functional expression of choroid plexus RNA injected into *Xenopus* oocytes (Lübbert et al., 1987; Julius et al., 1988). The genomic organization of the 5-HT$_{1C}$ receptor (with the presence of introns), a 50% overall homology to the 5-HT$_{2A}$ receptor and similar signal transduction mechanisms as 5-HT$_{2A}$ receptor and 5-HT$_{2B}$ receptor, indicated that the 5-HT$_{1C}$ receptor was more similar to members of the 5-HT$_2$ receptor family than the intronless, adenylyl cyclase–coupled members of

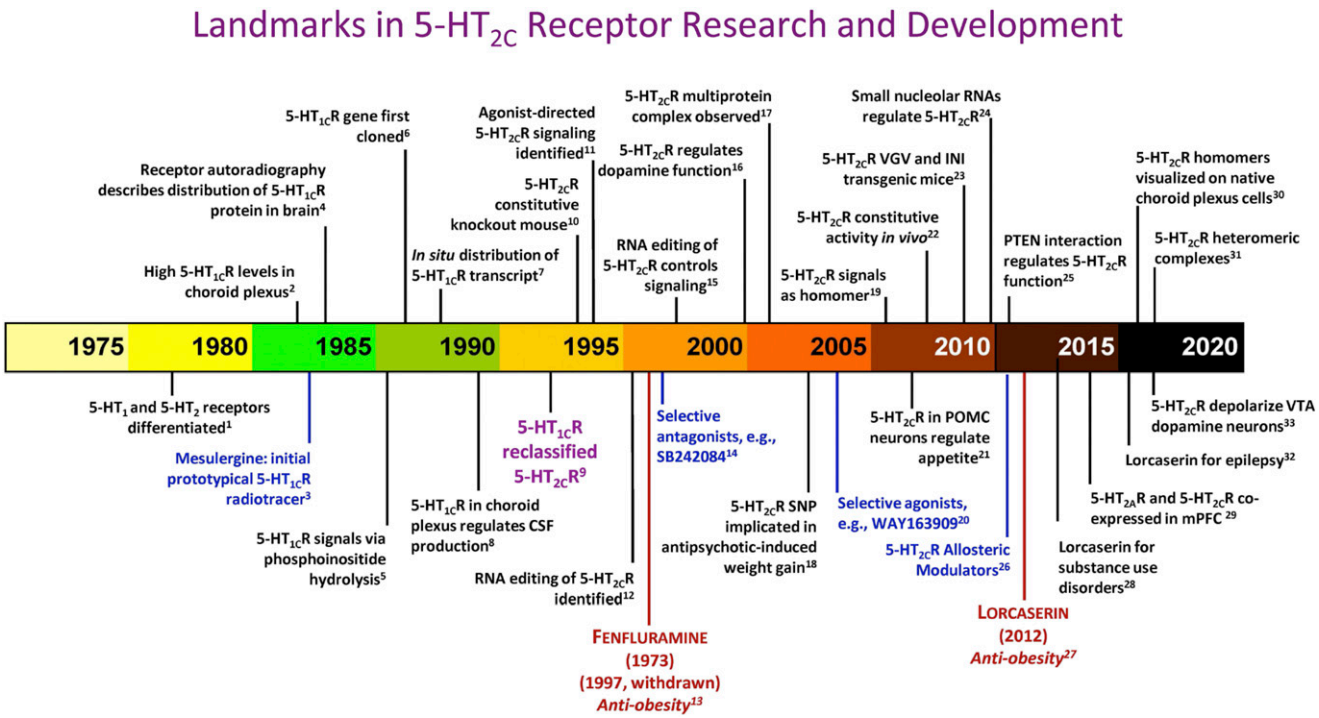

Fig. 12. Landmarks in 5-HT_{2C} receptor research and development. Identification and characterization of the 5-HT_{2C} receptor and the development of 5-HT_{2C} receptor–directed ligands has evolved over the last 40 years (black text). The 5-HT_{1C} receptor was reclassified as the 5-HT_{2C} receptor in the mid-1990s (purple text). Examples of novel pharmacological tools (blue text) as well as the first-in-class selective 5-HT_{2C} receptor agonist approved for obesity (red text) are illustrated in the timeline. Refer to the text for details; superscripted numbers indicate literature citations: 1) Peroutka and Snyder, 1979; 2) Pazos et al., 1984b; Yagaloff and Hartig, 1985; 3) Closse, 1983; 4) Pazos et al., 1984a; Pazos and Palacios, 1985; Pazos et al., 1985; Hoyer et al., 1986; 5) Conn et al., 1986; Conn and Sanders-Bush, 1986a; Hoyer et al., 1989; Chang et al., 2000; 6) (rat) Lübbert et al., 1987; Julius et al., 1988; (human) Saltzman et al., 1991; Milatovich et al., 1992; Stam et al., 1994; Xie et al., 1996; (mouse) Yu et al., 1991; Foguet et al., 1992a,b; 7) Hoffman and Mezey, 1989; Molineaux et al., 1989; Mengod et al., 1990; 8) Millar et al., 2007; 9) Hoyer, 1988a,b; Humphrey et al., 1993; Hoyer et al., 1994; 10) Tecott et al., 1995; 11) Berg et al., 1994, 1998a; 12) Burns et al., 1997; 13) http://www.fda.gov/Drugs/DrugSafety/PostmarketDrugSafetyInformationforPatientsandProviders/ucm180078.htm; 14) Bromidge et al., 1997; Kennett et al., 1997; 15) Herrick-Davis et al., 1999; 16) Di Giovanni et al., 1999; Di Matteo et al., 1999; Gobert et al., 2000; 17) Bécamel et al., 2002, 2004; 18) Basile et al., 2002; Tsai et al., 2002; Theisen et al., 2004; Templeman et al., 2005; 19) Herrick-Davis et al., 2006; Herrick-Davis et al., 2007; 20) Rosenzweig-Lipson et al., 2007a; Tong et al., 2010; Dunlop et al., 2011; Dunlop et al., 2005; 21) Xu et al., 2008 22) Leggio et al., 2009b; 23) Kawahara et al., 2008; Morabito et al., 2010a; 24) Kishore and Stamm, 2006; Doe et al., 2009; Kishore et al., 2010; 25) Ji et al., 2006; Anastasio et al., 2013; 26) Im et al., 2003; Ding et al., 2012; Wild et al., 2019; 27) http://www.fda.gov/NewsEvents/Newsroom/PressAnnouncements/ucm309993.htm; Thomsen et al., 2008; Smith et al., 2009; Smith et al., 2010; 28) http://www.eisai.com/news/news201465.html; Cunningham and Anastasio, 2014; Rezvani et al., 2014; Higgs et al., 2015; Howell and Cunningham, 2015; Harvey-Lewis et al., 2016; 29) Nocjar et al., 2015; Anastasio et al., 2015; 30) Herrick-Davis et al., 2015; 31) Schellekens et al., 2015; Kamal et al., 2015; 32) Di Giovanni and De Deurwaerdere, 2016; Venzi et al., 2016; 33) Xu et al., 2017.

the 5-HT_1 receptor family. Therefore, the 5-HT_{1C} receptor was reclassified as a member of the 5-HT_2 receptor family and renamed 5-HT_{2C} receptor (Hoyer et al., 1994).

Early autoradiography studies reported a predominant localization of this receptor in choroid plexus tissue, whereas subsequent in situ hybridization studies revealed a widespread distribution throughout the basal ganglia, limbic system, and prefrontal cortex (Hoyer et al., 1986; Hoffman and Mezey, 1989; Molineaux et al., 1989; Mengod et al., 1990; Fig. 13). In fact, 5-HT_{2C} receptor mRNA has been reported to be more abundant and widespread throughout the CNS than mRNA of the closely related 5-HT_{2A} receptor (Pompeiano et al., 1994; Wright et al., 1995). Thus, the 5-HT_{2C} receptor, largely thought of as "the choroid plexus receptor," is actually well positioned throughout the CNS to mediate many of the central actions of 5-HT, including regulation of appetite, cognition, mood, movement, and sleep [for reviews, see Berg et al. (2008a) and Di Giovanni et al., 2010)]. The 5-HT_{2C} receptor has been implicated in addiction, anxiety, depression, epilepsy, schizophrenia, and obesity. Therefore, the 5-HT_{2C} receptor is a therapeutic target of great interest [for reviews, see Bubar and Cunningham (2006); Howell and Cunningham (2015); Sullivan et al. (2015); Di Giovanni and De Deurwaerdere (2016)].

B. Expression Profile

The 5-HT_{2C} receptor mRNA is differentially overexpressed in neurons relative to astrocytes and oligodendrocytes in the postnatal mouse forebrain (Cahoy et al., 2008). Regional analyses indicate that the 5-HT_{2C} receptor mRNA is present in high levels in the choroid plexus, hippocampus, and the subthalamic and lateral habenular nuclei (Hoffman and Mezey, 1989; Molineaux et al., 1989; Mengod et al., 1990). Brain regions with moderate to high levels of 5-HT_{2C} receptor mRNA include the amygdala, nuclei of the basal ganglia (NAc, striatum, VTA, SN, and internal globus pallidus), cortex, hypothalamus, dorsal raphe, brain stem, and spinal cord (Hoffman and

Mezey, 1989; Molineaux et al., 1989; Mengod et al., 1990). The 5-HT_{2C} receptor mRNA is found in regions containing the major dopaminergic cell bodies, including the SN and the VTA (Mengod et al., 1990; Pompeiano et al., 1994; Eberle-Wang et al., 1997; Bubar and Cunningham, 2007; Bubar et al., 2011); cholinergic cell bodies (Lopez-Gimenez et al., 2001); encephalin-, substance P–, and dynorphin-containing neuropeptidergic neurons in the dorsal and ventral striatum (Ward and Dorsa, 1996); and neuropeptide Y–containing neurons in the lateral and basolateral amygdala (Bonn et al., 2013). The 5-HT_{2C} receptor mRNA is not present in neurons expressing 5-HT transporter (SERT) mRNA in the raphe nuclei but is localized to GABA interneurons in this region (Serrats et al., 2005).

There is good agreement between mRNA and protein distribution in the majority of brain regions, suggesting that the 5-HT_{2C} receptor is predominantly localized in somatodendritic compartments (Mengod et al., 1990; Abramowski et al., 1995; Abramowski and Staufenbiel, 1995; Eberle-Wang et al., 1997; Clemett et al., 2000; Anastasio et al., 2010). The exceptions are the subthalamic nucleus, in which 5-HT_{2C} receptor mRNA expression is high while its protein levels are low, and the external globus pallidus where the 5-HT_{2C} receptor is present but 5-HT_{2C} receptor mRNA is absent.

Early radioligand binding studies suggested that the 5-HT_{2C} receptor is located on the apical surface of epithelial cells in the choroid plexus (Hartig et al., 1990). More recently, confocal microscopy and fluorescence correlation spectroscopy were used to directly visualize native 5-HT_{2C} receptors and reveal their expression as homodimers on the apical surface of choroid epithelial cells (Herrick-Davis et al., 2015).

The 5-HT_{2C} receptor is widely distributed throughout the basal ganglia and limbic-corticostriatal circuit. The 5-HT_{2C} receptor is located postsynaptic to serotonergic neurons on GABAergic, glutamatergic, dopaminergic, neuropeptidergic, and cholinergic neurons. For example, the 5-HT_{2C} receptor is expressed on GABAergic interneurons in the raphe nuclei (Serrats et al., 2005) and on GABAergic projection neurons in the NAc and striatum (Alex and Pehek, 2007). They are also found on GABAergic neurons in the substantia nigra (Eberle-Wang et al., 1997; Invernizzi et al., 2007) and VTA (Di Giovanni et al., 2001; Bubar and Cunningham, 2007; Bubar et al., 2011). Because the 5-HT_{2C} receptor stimulates IP turnover (Conn and Sanders-Bush, 1986a; Hoyer et al., 1989; Chang et al., 2000) and increases Ca_i^{2+} levels resulting in membrane depolarization and neuronal firing (Stanford et al., 2005), activation of the 5-HT_{2C} receptor on GABAergic neurons would be expected to increase the firing of these neurons, which have an inhibitory influence in that region. For example, stimulation of the 5-HT_{2C} receptor in the VTA increases the firing rate of GABAergic interneurons, resulting in a decreased firing rate of dopaminergic neurons (Prisco et al., 1994; Di Giovanni et al., 2001). Conversely, 5-HT_{2C} receptor antagonism has been reported to increase dopaminergic neurotransmission and dopamine levels in the NAc and prefrontal cortex (Di Giovanni et al., 1999; Di Matteo et al., 1999; Gobert et al., 2000). However, the story is likely to be more complicated, as the 5-HT_{2C} receptor has also been shown to be expressed on a subset of dopaminergic neurons in the VTA, with higher expression in the middle relative to the rostral and caudal regions (Bubar and Cunningham, 2007; Bubar et al., 2011). These findings suggest the possibility that activation of the 5-HT_{2C} receptor may directly enhance dopamine neurotransmission within specific subnuclei of the VTA in a region-specific manner.

5-HT neurons from the raphe terminate in layers V and VI of the medial prefrontal cortex, adjacent to GABAergic interneurons that express the 5-HT_{2C} receptor (Liu et al., 2007; Nocjar et al., 2015). In turn, the GABAergic interneurons terminate on pyramidal efferents (likely glutamatergic), which may also express the

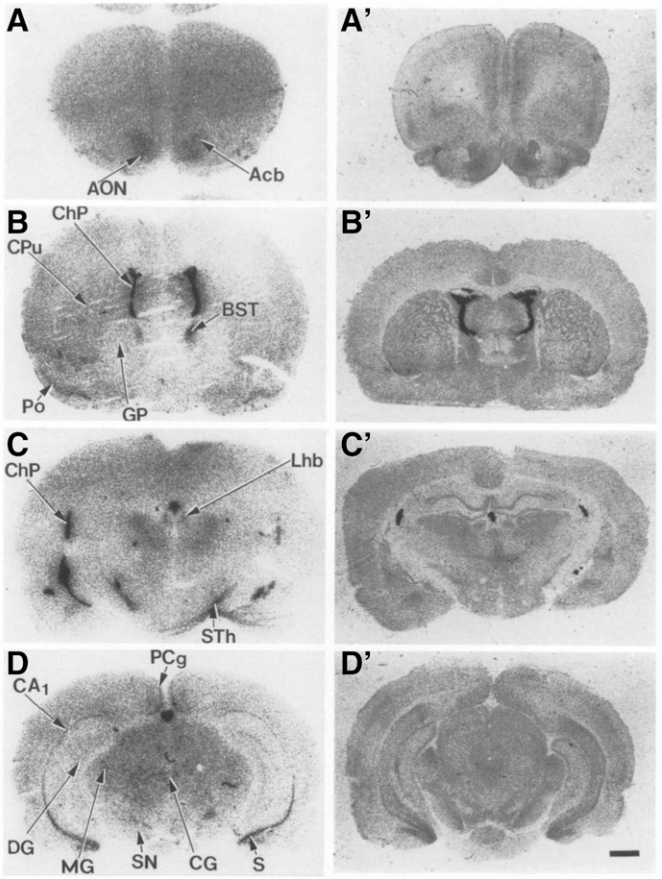

Fig. 13. 5-HT_{2C} receptor expression in rat brain. In situ hybridization detection of 5-HT_{2C} receptor mRNA (A–D) and 5-HT_{2C} receptor autoradiography (A'–D') from adjacent sections. Acb, nucleus accumbens; AON, anterior olfactory nucleus; BST, bed nucleus stria terminalis; CA1, hippocampus CA1 field; CG, central gray; ChP, choroid plexus; CPu, caudate-putamen; DG, dentate gyrus; GP, globus pallidus; Lhb, lateral habenular nucleus; MG, medial geniculate nucleus; PCg, posterior cingulate cortex; PO, primary olfactory cortex; S, subiculum; SN, substantia nigra; STh, subthalamic nucleus. Scale bar, 1 mm. Adapted from Mengod et al. (1990) (with permission).

5-HT_{2C} receptor (Vysokanov et al., 1998; Carr et al., 2002; Liu et al., 2007; Nocjar et al., 2015). In the prefrontal cortex, the 5-HT_{2C} receptor associates with PSD-95 in postsynaptic densities (Anastasio et al., 2010) and colocalizes with the 5-HT_{2A} receptor on GABAergic interneurons (Nocjar et al., 2015). In the hypothalamus, the 5-HT_{2C} receptor is expressed on pro-opiomelanocortin (POMC) neurons in the arcuate nucleus, where they stimulate the release of the anorectic peptide α-melanocyte–stimulating hormone (Heisler et al., 2002).

Previous studies provide little evidence that the 5-HT_{2C} receptor is expressed outside the CNS. However, portions of the *HTR2C* gene are expressed outside the CNS. Exons I and II of the *HTR2C* gene are located in the 5′ untranslated region, and the translational start site for the 5-HT_{2C} receptor protein is located in the middle of exon III (Xie et al., 1996). Exons I–III of the *HTR2C* gene have been reported to be expressed in non-neuronal cells, along with four different microRNAs (miRNA) generated from intron II (Zhang et al., 2013c). Expression of these miRNAs is regulated by the small nucleolar RNAs (snoRNA) SNORD 115/MBII-52 and SNORD 116/MBII-85. Patients with Prader-Willi syndrome lack MBII-52 and MBII-85 and express different levels of the 5-HT_{2C} receptor miRNAs than control subjects (Zhang et al., 2013c). Although the function of these miRNAs is not fully understood, it is possible that their dysregulation may contribute to the Prader-Willi syndrome phenotype as explored in 5-HT_{2C} receptor transgenic mouse models.

Because the 5-HT_{2C} receptor mRNA in human brain includes exons I–VI, and the mRNA found outside the CNS contains exons I–III, it was hypothesized that a transcriptional termination signal prior to exon IV may be employed in non-neuronal cells outside the CNS (Zhang et al., 2013c). In contrast, a 185-base-pair fragment of 5-HT_{2C} receptor mRNA was identified by quantitative RT-PCR from rat adipocyte visceral tissue (Stunes et al., 2011). The expression level of the 5-HT_{2C} receptor mRNA was only 5% of the expression level of the β-actin housekeeping gene control, and Western blots revealed that 5-HT_{2C} receptor expression in undifferentiated adipocytes was negligible compared with expression in rat brain (Stunes et al., 2011). However, expression of 5-HT_{2C} receptor mRNA and protein expression could be induced in cultured adipocytes. In addition, low levels of 5-HT_{2C} receptor mRNA and protein have been reported in rat pancreatic islet cells (Zhang et al., 2013b). Further research is required to elucidate the physiologic role of the 5-HT_{2C} receptor in the function of adipocytes (Stunes et al., 2011) and potentially pancreatic β-cells (Zhang et al., 2013b).

C. Post-transcriptional and Post-translational Modifications

1. *RNA* Editing. The 5-HT_{2C} receptor is the only GPCR reported to undergo RNA editing. The 5-HT_{2C} receptor pre-mRNA is unique in containing a region of exonic and intronic sequence complementarity between the distal half of exon V and the beginning of intron V (Burns et al., 1997). This results in base pairing between the exonic and intronic sequences, giving the pre-mRNA a double-stranded, stem-loop structure. The secondary structure of the 5-HT_{2C} receptor pre-mRNA influences the pattern of editing (Fukuda et al., 2015) as well as splicing (Shen et al., 2013). The double-stranded RNA is a substrate for adenosine deaminases that act on RNA and RNA-specific adenosine deaminase (ADAR) 1 and ADAR2 (Burns et al., 1997; Liu et al., 1999). These enzymes catalyze the deamination of adenosine to inosine, which is read as guanosine when the RNA is translated into protein. Adenosine to inosine RNA editing can occur at up to five different locations (termed A–E) within exon V (Burns et al., 1997; Fitzgerald et al., 1999; Niswender et al., 1999; Wang et al., 2000a) and one site located in intron V (Flomen et al., 2004). The editing sites in exon V are located in the second intracellular loop of the receptor in close proximity to the highly conserved "DRY" motif at the base of transmembrane domain III. The resulting adenosine to guanosine conversions change the coding potential of amino acids I156, N158, and I160 from INI (isoleucine, asparagine, isoleucine) in the unedited isoform to VSV (valine, serine, valine) or VGV (valine, glycine, valine) in the fully edited isoforms.

The RNA editing of the *HTR2C* gene can produce up to 32 different 5-HT_{2C} receptor pre-mRNAs encoding 24 different proteins. There are seven predominant 5-HT_{2C} receptor isoforms that are expressed in a region-specific manner in human and rodent brain (Burns et al., 1997; Fitzgerald et al., 1999; Niswender et al., 1999; Wang et al., 2000; Dracheva et al., 2009; Abbas et al., 2010; Morabito et al., 2010b). The more highly edited isoforms are the predominant isoforms expressed in whole brain, with VSV and VNV being the most prominent. Regions such as the hypothalamus, hippocampus, striatum, and cortex predominantly express the edited VNV, VSV, and VSI isoforms, whereas the choroid plexus and cerebellum express significantly higher levels of the unedited INI and partially edited INV and ISV isoforms.

Because RNA editing occurs in the second intracellular loop of the receptor, a region known to play an important role in G protein activation, it has profound effects on the pharmacology and signaling capabilities of the 5-HT_{2C} receptor. RNA editing decreases agonist binding affinity and G protein coupling efficiency (Burns et al., 1997; Fitzgerald et al., 1999; Herrick-Davis et al., 1999; Niswender et al., 1999; Wang et al., 2000; Berg et al., 2001). In these studies, the fully edited VSV and VGV isoforms displayed a 5- to 40-fold decrease in 5-HT binding affinity and potency for stimulating phosphoinositide production compared with the unedited INI isoform, and they displayed decreased affinity and potency for a variety

of different agonists. When expressed in COS-7 cells at levels similar to native 5-HT_{2C} receptor in choroid epithelial cells, the unedited INI isoform displayed high constitutive activity that was successively diminished by RNA editing to very low levels of constitutive activity in the fully edited VSV and VGV isoforms (Herrick-Davis et al., 1999; Niswender et al., 1999). In addition to regulating the level of basal activity, RNA editing alters the specificity of G protein coupling and second messenger activation. For example, the unedited INI isoform can signal through G_q, G_{13}, and G_{15}, but the fully edited VSV and VGV isoforms predominantly couple to and signal through G_q (Price et al., 2001; McGrew et al., 2002, 2004). The 5-HT_{2C} receptor has been shown to signal in an agonist-specific manner to differentially regulate phosphoinositide and arachadonic acid production (Berg et al., 1998b) and the phosphorylation of $ERK_{1/2}$ (Werry et al., 2005), effects which are diminished following RNA editing (Berg et al., 2001, 2008b; Werry et al., 2008). In addition to impacting signaling, RNA editing also influences receptor desensitization and trafficking (Marion et al., 2004).

The 5-HT_{2C} receptor pre-mRNA editing is regulated by ADAR1 and ADAR2 (Burns et al., 1997). Both in vitro and in vivo studies have reported that RNA editing at amino acid 156 of the 5-HT_{2C} receptor is predominantly accomplished by ADAR1, editing at amino acid 160 requires ADAR2, and both enzymes participate in editing at amino acid 158 (Liu et al., 1999; Wang et al., 2000, 2004; Hartner et al., 2004). ADAR1 and ADAR2 may act in a concerted manner whereby ADAR1 editing at amino acid 156 enhances ADAR2 pre-mRNA binding and subsequent editing at amino acids 160 and 158 (Carmel et al., 2012). In addition, factors that regulate the alternative splicing of the ADAR1 and ADAR2 pre-mRNAs have been reported to influence 5-HT_{2C} receptor RNA editing (Liu et al., 1999; Schmauss et al., 2010). Alterations in ADAR activity or pattern of expression can result in altered 5-HT_{2C} receptor isoform expression. Studies using neuronal cell cultures showed that factors that increase ADAR1 expression, such as treatment with interferon, increase 5-HT_{2C} receptor editing at amino acid 156 (Yang et al., 2004) and that reduced ADAR1 expression abolished editing at amino acid 156 (Sukma et al., 2005). In vivo evidence supporting this hypothesis is provided by a recent study reporting a downregulation in ADAR2 expression following spinal cord injury in rats, which resulted in decreased 5-HT_{2C} receptor RNA editing at amino acid 160 (Di Narzo et al., 2015). Conversely, RNA editing has been reported to be increased in transgenic mice overexpressing ADAR2, which resulted in an increase in the expression of the more fully edited, and a decrease in expression of the unedited, 5-HT_{2C} receptor isoforms (Singh et al., 2011). The results of these studies indicate that alterations in ADAR activity and expression patterns alter the degree and pattern of 5-HT_{2C} receptor editing, resulting in changes in 5-HT_{2C} receptor isoform expression and signal transduction.

2. *RNA* Splicing. Three different variants of the 5-HT_{2C} receptor are produced by RNA splicing, only one of which is expressed on the plasma membrane and is fully functional. The full-length functional 5-HT_{2C} receptor (2Cfl) is generated following splicing at the traditional exon/intron boundaries to remove introns III–V (Xie et al., 1996). There are two alternative donor splice sites flanking the traditional donor splice site located at the exon V/intron V boundary: one located in the middle of exon V (Canton et al., 1996; Xie et al., 1996) and an infrequently used site located within intron V (Wang et al., 2000; Flomen et al., 2004). The splice sites are conserved across rat, mouse, and human species. Splicing at either one of the alternative splice sites produces an RNA that encodes a prematurely truncated protein. Use of the donor splice site located in the middle of exon V results in the deletion of the last 95 nucleotides of exon V, including the RNA editing sites, and causes a frame shift mutation with stop codon. The result is a truncated protein (2Ctr) containing the N terminus and first three transmembrane domains followed by 96 unique amino acids (Canton et al., 1996; Xie et al., 1996; Wang et al., 2000). In human brain tissue, 2Ctr mRNA is found in all brain regions containing 2Cfl mRNA. Though Western blots of membrane extracts from 2Ctr-transfected NIH3T3 cells revealed immunoreactive bands the predicted size of 2Ctr protein, radioligand binding and phosphoinositide production were not observed in the transfected cells (Canton et al., 1996; Wang et al., 2000). The 2Ctr protein is not expressed on the plasma membrane but is retained within the endoplasmic reticulum where the 2Ctr can form heterodimers with 2Cfl (Herrick-Davis and Farrington, 2011; Martin et al., 2013). Thus, one possible function of 2Ctr is to regulate 5-HT_{2C} receptor signaling by forming heterodimers with 2Cfl and trapping 2Cfl in the endoplasmic reticulum (Herrick-Davis and Farrington, 2011; Martin et al., 2013).

Factors that influence splice site selection will impact 5-HT_{2C} receptor function and signaling by altering the relative balance between 2Cfl and 2Ctr within a given brain region. RNA editing and factors that influence RNA editing, such as ADAR1 and ADAR2 activity, have been shown to influence splice site selection (Rueter et al., 1999; Maas et al., 2001; Flomen et al., 2004; Tohda et al., 2004; Dracheva et al., 2008a). Increased editing of the 5-HT_{2C} receptor pre-mRNA, generating the more fully edited and less active isoforms, promotes the use of the traditional donor splice site at the exon V/intron V boundary and favors the generation of 2Cfl over 2Ctr (Flomen et al., 2004). On the other hand, the unedited and highly active INI isoform is associated with increased use of the alternative donor splice site in the middle of exon V, increasing the production of 2Ctr. The preferred splicing of the INI isoform into 2Ctr (Flomen et al., 2004) may explain the higher plasma

membrane expression levels of 2Cfl observed in transgenic mice expressing the VGV isoform compared with mice expressing the INI isoform of the 5-HT_2R (Kawahara et al., 2008). These results are consistent with the original studies in which 2Ctr was first identified and was reported to be most abundant in the choroid plexus (Canton et al., 1996; Xie et al., 1996), the same brain region that was subsequently reported to express the highest levels of unedited and partially edited isoforms of the 5-HT_{2C} receptor (Burns et al., 1997; Niswender et al., 1999; Wang et al., 2000).

Several studies have provided evidence for a link between 5-HT_{2C} receptor RNA editing and splicing in vivo. In postmortem brain samples from patients who committed suicide, RNA editing was increased along with the ratio of 2Cfl to 2Ctr, with increased expression of the less-active 5-HT_{2C} receptor isoforms (Dracheva et al., 2008a). In malignant gliomas from human brain, decreased ADAR2 activity (predicted to reduce RNA editing) was positively correlated with increased alternative splicing and the production of 2Ctr (Maas et al., 2001). In several of the gliomas examined and in glioma-derived cells lines, 2Ctr was predominant and 2Cfl was mostly absent (Maas et al., 2001; Tohda et al., 2004). In light of these findings, it is interesting to note that patients with glioblastoma have an increased incidence of seizures, as do 5-HT_{2C} receptor knockout mice.

noRNAs also regulate 5-HT_{2C} receptor RNA splicing. The snoRNA MBII-52 binds to exon V of the *HTR2C* gene in the region containing the alternative splice site and the RNA editing sites (Kishore and Stamm, 2006; Kishore et al., 2010). MBII-52 reduces RNA editing and the use of the alternative splice site, thereby favoring the production of the full-length, unedited, and more active 5-HT_{2C} receptor isoforms. In mice lacking MBII-52, RNA editing is increased, leading to increased expression of the edited and less-active 5-HT_{2C} receptor isoforms (Doe et al., 2009). These mice display phenotypic and behavioral changes similar to those observed in Prader-Willi syndrome (Doe et al., 2009). Consistent with these findings, transgenic mice expressing only the fully edited VGV isoform display characteristics of Prader-Willi syndrome (Morabito et al., 2010a). The human homolog HBII-52 has been shown to be absent in patients with Prader-Willi syndrome, and 5-HT_{2C} receptor RNA editing is increased in patients with Prader-Willi syndrome (Kishore and Stamm, 2006). These studies highlight the important roles that RNA editing and splicing play in the regulation of 5-HT_{2C} receptor activity and demonstrate how deregulation of this system can have profound phenotypic and behavioral consequences.

3. Single-Nucleotide Polymorphisms. The 5-HT_{2C} receptor not only achieves diversity through RNA editing and splicing but also through the incorporation of SNPs, substitutions of a novel nucleotide for a wild-type nucleotide, a common type of genetic variation (Wang et al., 1998). These SNPs can produce an unstable conformation that alters the protein structure of a GPCR and affects its ultimate functional properties (Wenkert et al., 1996). A number of *HTR2C* SNPs have been reported in the literature: three polymorphisms as well as a GT nucleotide repeat variation have been identified in the promoter (Xie et al., 1996); three polymorphisms have been reported within intronic regions (Gibson et al., 2004); one polymorphism has been reported in the coding region, resulting in the replacement of cysteine with serine at amino acid 23 (C23S) in the amino-terminus of the receptor (Lappalainen et al., 1995); and there is one polymorphism in the 3′ untranslated region (Song et al., 1999). Few studies are reported that have ascertained the impact of these *HTR2C* SNPs on function at either the cellular or whole organism level. The impact of the S23 variant, when expressed in insect cells, was reported to have slightly higher affinity for 5-HT than the C23 variant and to alter 5-HT_{2C} receptor desensitization/resensitization mechanisms (Okada et al., 2004; Walstab et al., 2011). However, this finding remains controversial, as other studies have reported no difference between the C23 and S23 variants with respect to agonist-binding affinity or potency, G protein coupling, constitutive activity, and homodimerization (Lappalainen et al., 1995; Fentress et al., 2005). Given suggestions that the C23 variant may have clinical implications (Lappalainen et al., 1995; Okada et al., 2004; Piva et al., 2011; Walstab et al., 2011), further studies of this and other *HTR2C* SNPs are required.

4. Glycosylation. The rat 5-HT_{2C} receptor is reported to be glycosylated, and bands with different molecular weights were observed on Western blot of brain and cells expressing the receptor (Abramowski and Staufenbiel, 1995). Antibodies raised against the third and fourth cytoplasmic domain of the 5-HT_{2C} receptor identified an *N*-glycosylated polypeptide with a 60-kDa apparent molecular mass. Polypeptides were detected in immunoprecipitates from extracts of pig choroid plexus upon Western blot and binding assays with [^{3}H]-mesulergine. A signal sequence was cleaved during membrane insertion, resulting in a 38-kDa polypeptide. During further maturation, the receptor was *N*-glycosylated at two sites via a 48-kDa intermediate, which was more abundant in choroid plexus than in hippocampus. However, there may be more glycosylated species, as following transfection of 5-HT_{2C} receptor cDNAs into cultured cells, polypeptides were observed that differed from the ones found in the brain. Similarly, the *N*-glycosylated 5-HT_{2C} receptor was identified in solubilized extracts from cell lines and rat brain (Backstrom et al., 1995). Extracts from NIH3T3 fibroblasts stably expressing rat 5-HT_{2C} receptor contained immunoreactive proteins of 51 to 52 kDa and 58 to 68 kDa. In the brain, immunoreactive proteins were identified from choroid plexus extracts with masses of 51 kDa and

58–62 kDa. On the other hand, the major 58- to 62-kDa and minor 51-kDa proteins were not detected in extracts prepared from the hippocampus, striatum, or frontal cortex prepared under the same conditions. The association of asparagine-linked (N-linked) oligosaccharides with the receptors was also examined. Cells grown in the presence of tunicamycin to inhibit N-linked glycosylation resulted in proteins with masses of 40 and 41 kDa. Extracts prepared from NIH3T3 cells and choroid plexus incubated with N-glycosidase F showed proteins of 41 and 42 kDa from NIH3T3 cells and 41 kDa from choroid plexus. Neuraminidase treatment, to cleave sialic acid, reduced the mass of the 51-kDa and 58- to 62-kDa proteins from the choroid plexus to 50 kDa and 54–58 kDa, whereas the proteins from NIH3T3 cells were not affected by neuraminidase. Altogether, the 5-HT_{2C} receptor contains N-linked sugars, and it is suggested that sialic acid residues associate with the receptor from the choroid plexus but not from other brain regions. These oligosaccharide moieties contribute up to approximately 30% of the relative mass of the receptor and may affect the functional properties of the 5-HT_{2C} receptor.

5. Phosphorylation. The constitutively active 5-HT_{2C} receptor is phosphorylated under basal conditions, and phosphorylation is increased by agonist treatment (Westphal et al., 1995). Pretreatment of cells with 5-HT resulted in 5-HT_{2C} receptor desensitization, which was blocked by calcineurin (presumably by its phosphatase activity) (Boddeke et al., 1993). It was subsequently shown that S458 and S459 are phosphorylated (Backstrom et al., 2000). Phosphorylation of a mutant 5-HT_{2C} receptor that lacks the carboxyl-terminal PDZ recognition motif [Ser(458)-Ser-Val-COOH; δPDZ] was not detectable, although these cells produced similar amounts of phosphoinositide and Ca_i^{2+} with similar kinetics as wild-type cells. Alanine mutations S458A or S459A decreased phosphorylation to 50% of wild-type receptor levels. Subsequent Ca_i^{2+} responses of S459A receptors were diminished relative to S458A and wild-type receptors. Thus, desensitization may occur in the absence of 5-HT_{2C} receptor phosphorylation, suggesting that receptor phosphorylation at S459 enhances resensitization of 5-HT_{2C} receptor responses. Agonist-induced phosphorylation of the 5-HT_{2C} receptor was later established to regulate the receptor interaction with multiple PDZ protein 1 (Parker et al., 2003), also known as MUPP1 (Ullmer et al., 1998). MUPP1 is a putative scaffolding protein containing 13 PSD-95, Dlg, ZO-1 (PDZ) domains, identified by a yeast two-hybrid screen as one of a series of 5-HT_{2C} receptor–interacting proteins. The MUPP1 PDZ domain 10 (PDZ 10) associates with Ser458-Ser-Val of the 5-HT_{2C} receptor. An Asp mutation at Ser458 significantly decreased receptor interaction with PDZ 10. Also, 5-HT treatment of 5-HT_{2C} receptor–NIH3T3 cells reduced receptor interaction with PDZ 10, an effect that was blocked by a 5-HT_{2C} receptor antagonist.

Phosphatase and tensin homolog deleted on chromosome 10 (PTEN) interacts within several amino acids of the third intracellular loop (termed 3L4F) of the 5-HT_{2C} receptor (Ji et al., 2006). The tumor suppressor PTEN is widely distributed in the brain (Lachyankar et al., 2000). The PTEN and 5-HT_{2C} receptor proteins coimmunoprecipitate in fractions of the VTA (Ji et al., 2006; Anastasio et al., 2013). Employing a proximity ligation assay, the assembly of the 5-HT_{2C} receptor:PTEN complex, and the ability of 3L4F to disrupt the complex, was validated under native conditions within intact live cells (Anastasio et al., 2013). A peptide fragment of the 5-HT_{2C} receptor third intracellular loop (3L4F), labeled with the cell-penetrating peptide TAT, disrupts the 5-HT_{2C} receptor:PTEN complex and PTEN-mediated dephosphorylation of the 5-HT_{2C} receptor in PC12 cells (Ji et al., 2006) as well as enhances 5-HT–mediated i Ca_i^{2+} release in CHO cells stably transfected with the human 5-$HT_{2C\text{-}INI}$ receptor (but not the 5-HT_{2A} receptor) (Anastasio et al., 2013). Interestingly, the cell-adhesion molecule close homology of L1 protein also binds to the third intracellular loop of the 5-HT_{2C} receptor (amino acids 292–304) and may regulate the 5-HT_{2C} receptor association with PTEN and β-$arrestin_2$ to control its phosphorylation (Kleene et al., 2015).

Systemic administration of the TAT-3L4F peptide suppresses the Δ^9-tetrahydrocannabinol (THC)-induced increase in firing rate of VTA dopaminergic neurons in the rat (Ji et al., 2006) and suppresses the place association conditioned to Δ^9-THC and nicotine in rats (Ji et al., 2006). In more recent studies, whereas TAT-3L4F had no effect alone, the combination of ineffective doses of TAT-3L4F plus WAY163909 synergize to suppress motor impulsivity, a primary symptomatic element of multiple neuropsychiatric disorders (American Psychiatric Association, 2013) that is consistently suppressed by pretreatment with a selective 5-HT_{2C} receptor agonist (Winstanley et al., 2004; Fletcher et al., 2007; Fletcher et al., 2011; Anastasio et al., 2013; Cunningham et al., 2013). In addition, TAT-3L4F augments 5-HT_{2C} receptor agonist–mediated suppression of spontaneous locomotor activity, effects that are consistent with positive allosteric modulation of 5-HT_{2C} receptor function (Anastasio et al., 2013; Wild et al., 2014, 2019). Together, coupled with the findings that Tat-3L4F did not affect spatial learning and memory (Maillet et al., 2008) or generate the total behavioral profile of 5-HT_{2C} receptor agonists (Ji et al., 2006; Anastasio et al., 2013), inhibition of the PTEN:5-HT_{2C} receptor interface and, thus, 5-HT_{2C} receptor dephosphorylation is a novel pharmacological approach with therapeutic potential in substance use disorders (Ji et al., 2006; Muller and Carey, 2006; Maillet et al., 2008; Anastasio et al., 2013; Cunningham and Anastasio, 2014).

6. Dimerization. Homodimerization for many GPCRs is thought to be a post-translational event that occurs within the endoplasmic reticulum as a prerequisite for the transport and expression of functional receptors on the

plasma membrane [for review, see Milligan (2010)]. Many members of the 5-HT receptor family, including the 5-HT_{2C} receptor, have been reported to form homodimers [for review, see Herrick-Davis (2013)]. Using a confocal microscopy-based resonance energy-transfer technique, 5-HT_{2C} receptor homodimer formation was visualized within the endoplasmic reticulum during receptor biosynthesis (Herrick-Davis et al., 2006). The 5-HT_{2C} receptor is transported through the Golgi complex to the plasma membrane as a homodimer.

The 5-HT_{2C} receptor forms detergent-sensitive homodimers (Herrick-Davis et al., 2004) that do not dissociate or associate to form higher-order complexes following agonist or inverse agonist treatment (Herrick-Davis et al., 2007). Coexpression of wild-type receptors with inactive, mutant receptors provided evidence that the 5-HT_{2C} receptor homodimer interacts with a single G protein, that both protomers participate in signaling, and that both protomers must be functional in order for signaling to occur (Herrick-Davis et al., 2005). Time-lapse fluorescence confocal microscopy provided direct visualization of β-$arrestin_2$ recruitment to the plasma membrane following 5-HT binding to the homodimer (Herrick-Davis et al., 2007; Herrick-Davis et al., 2012). Homodimerization was observed for both the unedited INI and the fully edited (VSV and VGV) isoforms (Herrick-Davis et al., 2007; Herrick-Davis et al., 2012).

Advanced imaging techniques with near-single-molecule sensitivity have been employed more recently in an attempt to distinguish between dimers and higher-order oligomers. Fluorescence correlation spectroscopy studies report that the 5-HT_{2C} receptor expressed in HEK293 cells exists as homodimers, with no evidence for monomers, tetramers, or higher-order oligomers (Herrick-Davis et al., 2012). Spatial intensity distribution analysis was used to monitor the oligomer status of the 5-HT_{2C} receptor over a wide range of receptor-expression levels (Ward et al., 2015). In this study, the 5-HT_{2C} receptor was expressed as a mixture of monomers, dimers, and tetramers; treatment with a 5-HT_{2C} receptor antagonist for 90 minutes converted the majority of dimers and tetramers to monomers. However, it should be noted that tetramers were prominent only when 5-HT_{2C} receptor expression exceeded 100 receptors/μm^2 (Ward et al., 2015), greatly in excess of physiologic expression levels for native GPCRs (Hegener et al., 2004; Herrick-Davis et al., 2015). Similarly, the homodimer was the predominant species observed for biogenic amine receptors when expressed within their normal physiologic range in HEK293 cells (Herrick-Davis et al., 2013).

Only one study to date has examined the 5-HT_{2C} receptor endogenously expressed in its native cellular environment. The native 5-HT_{2C} receptor, endogenous to the choroid plexus, is expressed as homodimers on the apical surface of the epithelial cells at a density of 32 receptors/μm^2 (Herrick-Davis et al., 2015). Though this is similar to a reported density of 20 receptors/μm^2 for native β_2-adrenergic receptors in alveolar epithelial cells, expression levels in neurons were much lower at 4.5 receptors/μm^2 (Hegener et al., 2004). The study by Herrick-Davis et al. (2015) found no evidence for monomers or tetramers of the native 5-HT_{2C} receptor in choroid epithelial cells or when the 5-HT_{2C} receptor was expressed at normal physiologic levels in HEK293 cells. In this study, the signaling properties of the 5-HT_{2C} receptor homodimer were investigated using agonists that bind in a wash-resistant manner to one or both protomers of the 5-HT_{2C} receptor homodimer. Agonist binding to one protomer stimulated a half-maximal phosphoinositide response, whereas binding to both protomers was required to produce a maximal response (Herrick-Davis et al., 2015). These experiments provide pharmacological evidence supporting the hypothesis that the 5-HT_{2C} receptor functions as a homodimer.

Heterodimers can form between the different isoforms of the 5-HT_{2C} receptor generated by RNA editing (Herrick-Davis and Farrington, 2011). In HEK293 cells, positive resonance energy transfer was observed between the INI/VSV, INI/VGV, and VSV/VGV isoform pairs. However, the influence of heteromer pairs of edited 5-HT_{2C} receptor isoforms on receptor signaling and neural function in vivo are yet to be demonstrated. In terms of heterodimers between the 5-HT_{2C} receptor and other members of the 5-HT receptor family, the 5-HT_{2A} receptor would be the most likely candidate given the similarity in structure (for review, see Hannon and Hoyer, 2008a,b). Immunohistochemical analyses indicate that the 5-HT_{2A} receptor and 5-HT_{2C} receptor protein colocalize in the same GABAergic neurons as well as in a population of pyramidal projection neurons in the rat medial prefrontal cortex (mPFC) (Nocjar et al., 2015). Coimmunoprecipitation studies suggest that the 5-HT_{2A} receptor and 5-HT_{2C} receptor are found in the same protein complex in the rat mPFC (Anastasio et al., 2015). Further analyses of the structural and biologic significance of a possible heteromeric protein complex incorporating both the 5-HT_{2A} receptor and 5-HT_{2C} receptor are required to understand its potential role in behavior and neuropsychiatric disorders.

Selective 5-HT_{2A} receptor antagonists and selective 5-HT_{2C} receptor agonists have been noted to suppress a wide range of behaviors that are particularly well studied in preclinical models of addictive disorders (Bubar and Cunningham, 2008; Cunningham and Anastasio, 2014; Howell and Cunningham, 2015). The combination of low doses of the selective 5-HT_{2A} receptor antagonist M100907 plus the preferential 5-HT_{2C} receptor agonist MK212 evoked modest effects but resulted in an approximately additive suppression of cocaine-evoked hyperlocomotion and Fos expression in the caudate putamen (Pockros et al., 2012). In a second study, the combination of subthreshold doses of M100907 plus the selective 5-HT_{2C} receptor agonist WAY163909

synergistically suppressed inherent and cocaine-evoked motor impulsivity as well as cocaine-induced hyperactivity and cocaine-seeking behavior (Cunningham et al., 2013). These data raise the possibility that the 5-HT_{2A} receptor and 5-HT_{2C} receptor may act in concert to regulate the neural bases for behavior. Further analyses of the structural and functional interactions between the 5-HT_{2A} receptor and 5-HT_{2C} receptor are necessary to disentangle the manner in which these GPCRs interact at neuronal and circuit levels.

Microinfusion of the preferential 5-HT_{2A} receptor agonist DOI into the mPFC enhances (Wischhof et al., 2011), whereas intra-mPFC infusion of M100907 (Winstanley et al., 2003) suppresses, motor impulsivity. The density of 5-HT_{2A} receptor (Fink et al., 2015) as well as 5-HT_{2C} receptor protein expression in the mPFC (Anastasio et al., 2014b) predicts the level of motor impulsivity in outbred rats. Highly impulsive rats exhibit a greater 5-HT_{2A} receptor–mediated head twitch response and are more sensitive to the suppressive effects of the selective 5-HT_{2A} receptor antagonist M100907 on motor impulsivity (Fink et al., 2015). The levels of 5-HT_{2A} receptor and 5-HT_{2C} receptor protein predicted the intensity of motor impulsivity, and the ratio of the 5-HT_{2A} receptor to 5-HT_{2C} receptor protein in mPFC positively correlated with levels of motor impulsivity in individual outbred rats (Anastasio et al., 2015). High phenotypic motor impulsivity was associated with a diminished mPFC synaptosomal 5-HT_{2A}R:5-HT_{2C} receptor protein:protein interaction assessed by coimmunoprecipitation (Anastasio et al., 2015). Knockdown of 5-HT_{2C} receptor in the mPFC resulted in increased motor impulsivity and triggered a compensatory upregulation of 5-HT_{2A} receptor protein expression in mPFC and a leftward shift in the potency of M100907 to suppress impulsive behavior (Anastasio et al., 2015). These data further support the concept that an interactive relationship between the mPFC 5-HT_{2A} receptor and 5-HT_{2C} receptor is behaviorally relevant. The manner in which a potential 5-HT_{2A} receptor and 5-HT_{2C} receptor heteromeric protein complex in the mPFC contributes to high levels of inherent motor impulsivity remains to be uncovered.

The 5-HT_{2C} receptor has also been reported to form heterodimers with ghrelin GHS-R1a receptor when overexpressed in HEK293 cells and to colocalize with the GHS-R1a in cultured primary hypothalamic and hippocampal neurons from the rat (Schellekens et al., 2015). In this study, activation and blockade of 5-HT_{2C} receptor in vivo attenuated and potentiated, respectively, the orexigenic effects of ghrelin. Heterodimers between 5-HT_{2C} receptor and melatonin MT_2 receptors have also been reported in transfected cells as well as human cortex and hippocampus (Kamal et al., 2015). Interestingly, the novel antidepressant agomelatine demonstrates biased signaling and displays 5-HT_{2C} receptor and MT_2 receptor agonist properties, suggesting the heterodimer as a potential target for the development of a novel class of therapeutics for the treatment of psychiatric disorders as well as eating disorders and obesity.

Immunohistochemical studies demonstrated that the NMDA receptor GluN2A colocalizes with the 5-HT_{2C} receptor in rat spinal cord neurons, whereas coimmunoprecipitation analysis of synaptosomal fractions suggests formation of a 5-HT_{2C} receptor and GluN2A protein complex (Bigford et al., 2012). Stimulation of the 5-HT_{2C} receptor enhanced NMDA-evoked motoneuron depolarization through involvement of Src tyrosine kinase (Bigford et al., 2012). These results support the assembly of a functionally relevant 5-HT_{2C} receptor and NMDA receptor complex in the spinal cord, although the presence of this complex in the brain and its involvement in higher-order neural function is unexplored.

D. Pharmacology

For the 5-HT_{2C} receptor, the identification of selective orthosteric ligands relative to the close family members 5-HT_{2A} receptor and 5-HT_{2B} receptor is challenged by their ~50% overall homology, which rises to ~80% in the transmembrane domains, comprising the orthosteric receptor–binding pocket (Hoyer et al., 2002). Because of this challenge, the earliest preclinical and clinical research employed "preferential" 5-HT_{2C} receptor ligands, which frequently displayed affinity (agonists, antagonists) and/or efficacy (agonists) at the 5-HT_{2A} receptor and 5-HT_{2B} receptor. Because of the lack of selectivity of available agonists [e.g., MK212 and *m*-chlorophenylpiperazine (mCPP)] and antagonists (e.g., ketanserin), experimental outcomes with such compounds initially led to ambiguous conclusions concerning the biologic roles for the 5-HT_{2C} receptor, particularly in vivo. Furthermore, with the understanding that 5-HT_{2A} receptor or 5-HT_{2B} receptor agonists may evoke hallucinations (Nichols, 2004) or cardiac valvulopathy (Fitzgerald et al., 2000; Roth, 2007), respectively, the need for 5-HT_{2C} receptor orthosteric agonists that lack demonstrable efficacy at 5-HT_{2A} receptor or 5-HT_{2B} receptor is recognized. In 1997, the chemists at SmithKline Beecham synthesized and characterized SB242084 as the first selective 5-HT_{2C} receptor antagonist (Bromidge et al., 1997; Kennett et al., 1997). Because the 5-HT_{2C} receptor exhibits constitutive activity dependent on the edited isoform and the in vitro and in vivo conditions employed for analyses, SB242084 and other compounds in this series (e.g., SB206553 and SB243213) (Bromidge et al., 1997; Kennett et al., 1997) have been noted to act as inverse agonists to attenuate constitutive 5-HT_{2C} receptor activity (for reviews, see Aloyo et al., 2009; Sullivan et al., 2015). Atypical antipsychotic drugs are also reported to act as inverse agonists with the ability to inhibit 5-HT_{2C} receptor constitutive activity (Herrick-Davis et al., 2000; Rauser et al., 2001) and Spampinato and colleagues have identified the role

of 5-HT_{2C} receptor constitutive activity in the control of dopamine corticoaccumbens function (Aloyo et al., 2009; Leggio et al., 2009b).

A body of knowledge has been developed around several useful 5-HT_{2C} receptor agonists that have been available to scientists for the last 10 years. The compound RO60-0175 (Knight et al., 2004) has generated a great deal of information concerning the biologic role of the 5-HT_{2C} receptor (Millan et al., 1998; Di Matteo et al., 2000; Grottick et al., 2000; Filip and Cunningham, 2002; Tomkins et al., 2002; Leggio et al., 2009a; Fletcher et al., 2012). RO60-0175 exhibits affinity and efficacy for all three 5-HT_2 receptor subtypes, although many of its effects in vivo are blocked by SB242084 (Martin et al., 1998; Porter et al., 1999; Knight et al., 2004). Lorcaserin is a selective, high-efficacy 5-HT_{2C} receptor agonist that was marketed as Belviq for weight reduction in patients with a body mass index >30 or with a body mass index >27 comorbid with type 2 diabetes, hypertension, or dyslipidemia (www.us.eisai.com/). The availability of lorcaserin for clinical research has prompted a growing number of studies, particularly focused on addictive disorders (Rezvani et al., 2014; Higgs et al., 2016; Harvey-Lewis et al., 2016; for review, see Higgins et al., 2020). The preliminary results of a clinical trial (http://www.eisai.com/news/news201465.html) demonstrated the efficacy of lorcaserin to increase abstinence from nicotine, a highly abused psychostimulant, whereas preclinical studies continue to promote the prospects of lorcaserin as a tool to reduce substance use disorders (Levin et al., 2011; Cunningham and Anastasio, 2014; Rezvani et al., 2014; Howell and Cunningham, 2015; Harvey-Lewis et al., 2016; Higgins et al., 2012; for review, see Higgins et al., 2020). Strikingly, Di Giovanni's group has also recently shown that lorcaserin is very effective as an anticonvulsant in both animal models of generalized nonconvulsive absence epilepsy and temporal lobe epilepsy (Orban et al., 2014; Venzi et al., 2016).

A compound series developed at Wyeth Research includes vabicaserin (SCA-136), which acts as a selective 5-HT_{2C} receptor full agonist (K_i = 3 nM; efficacy 100% relative to 5-HT), a 5-HT_{2B} receptor antagonist (IC_{50} = 29 nM), and a weak 5-HT_{2A} receptor antagonist (IC_{50} = 1650 nM) (Rosenzweig-Lipson et al., 2007a; Tong et al., 2010; Dunlop et al., 2011). A randomized, double-blind, placebo-controlled study suggested the efficacy, safety, and tolerability of vabicaserin in the treatment of acute schizophrenia (Shen et al., 2014), although few preclinical analyses of this compound are published. WAY163909 is chemically similar to vabicaserin with high affinity (K_i = 10.5 nM) and full efficacy (90% relative to 5-HT) at the 5-HT_{2C} receptor. WAY163909 exhibits a lower affinity (K_i = 212 nM) and no efficacy at the 5-HT_{2A} receptor and is a weak partial agonist at the 5-HT_{2B} receptor (Dunlop et al., 2005). WAY163909 has been employed as a tool compound to test hypotheses related to the involvement of the 5-HT_{2C} receptor in animal models of addiction, depression, impulsivity, and schizophrenia (Dunlop et al., 2006; Marquis et al., 2007; Rosenzweig-Lipson et al., 2007b; Navarra et al., 2008; Cunningham et al., 2011, 2013; Anastasio et al., 2013; Navailles et al., 2013b; Anastasio et al., 2014a). More recently, rational medicinal chemistry approaches have been employed to craft novel, highly selective 5-HT_{2C} receptor agonists (Storer et al., 2014; Rouquet et al., 2015; Cheng et al., 2016). Some of these newer 5-HT_{2C} receptor agonists have been made commercially available (e.g., PF-3246799 and PF-4479745 from Pfizer) (Storer et al., 2014), which will allow their increasing employment in in vitro and in vivo studies and provide greater breakthroughs in our understanding of 5-HT_{2C} receptor biology.

The orthosteric site of a GPCR at which the endogenous agonist binds has been the traditional target for ligand discovery, but the chemical space for GPCR neuroprobes and therapeutics has greatly expanded with the discovery of allosteric ligands for many GPCR subfamilies. An allosteric modulator is a ligand that binds to a spatially distinct allosteric site and alters the receptor conformation to modulate its interaction with other ligands and/or signal transduction molecules [for reviews, see Conn et al. (2009) and Christopoulos et al. (2014)]. Allosteric sites are expected to exhibit higher sequence divergence across receptor subtypes relative to the highly conserved orthosteric domain (Kenakin, 2009; Kenakin and Miller, 2010). For example, a positive allosteric modulator (PAM) can increase the affinity and/or efficacy of the orthosteric ligand (Conn et al., 2009) and, thus, has the potential to improve its therapeutic index and diminish negative side effects. Such allosteric modulation is saturable (comes to a finite magnitude when the allosteric site is fully occupied) and probe-dependent (varies dependent on the orthosteric ligand) with the prospects for separate control of affinity and efficacy, making allosteric ligands intriguing therapeutic chemical targets (Kenakin, 2010). At present, allosteric modulators are defined operationally as positive (PAM), negative allosteric modulators, or neutral allosteric ligands, with the possibility of the additional property of allosteric agonism (agonist effects consequent to binding to allosteric sites; Christopoulos et al., 2014).

Adron Harris and colleagues were the first to identify the effects of the fatty acid amide oleamide to positively modulate 5-HT_{2C} receptor–mediated activity in *Xenopus* oocytes (Huidobro-Toro et al., 1996a), and further analyses have identified oleamide as a member of a family of amphipathic lipid metabolites that allosterically promote 5-HT receptor signaling through other receptors (e.g., 5-HT_{2A} receptor and 5-HT_7 receptor; Thomas et al., 1997; Thomas et al., 1998; Alberts et al., 2001) but also exhibit a myriad of additional effects on receptor signaling (Leggett et al., 2004). In 2003,

chemical library screening at Pharmacia (now Pfizer) resulted in the discovery and characterization of PNU-69176E as a PAM highly selective for the 5-HT_{2C} receptor over the 5-HT_{2A} receptor, 5-HT_{2B} receptor, 5-HT_7 receptor, and dopamine receptors (Im et al., 2003). In 2012, Zhou and colleagues optimized the synthetic route to generate PNU-69176E and its diastereomer (Ding et al., 2012). A series of new molecules based on the 4-alkylpiperidine-2-carboxamide scaffold were designed, synthesized, and pharmacologically evaluated as 5-HT_{2C} receptor PAMs (Wild et al., 2019). Several analogs, potentiated 5-HT–evoked Ca_i^{2+} in 5-HT_{2C} receptor CHO cells but not in 5-HT_{2A} receptor CHO cells; one compound was further evaluated in vivo and exhibited a favorable overall pharmacokinetic and behavioral profile in rats (Wild et al., 2018). In addition, two predicted allosteric sites were identified by molecular docking to a 5-HT_{2C} receptor homology model (Wild et al., 2018). Taken together, these data provide proof of concept that allosteric modulation of 5-HT_{2C} receptor may be a viable strategy toward the discovery of novel neurotherapeutics. Recent preclinical indications of the efficacy of allosteric modulators in disease models, coupled with the launch of cinacalcet and maraviroc as the first marketed GPCR allosteric modulators, provide strong validation of the potential clinical utility of allosteric modulators (Conn et al., 2009). Ultimately, further analyses of novel allosteric modulators will improve understanding of 5-HT_{2C} receptor function and how allosteric modulators may provide gain (or loss) of function in this system.

E. Signal Transduction

Intracellular signaling cascades stimulated by the 5-HT_{2C} receptor have been assessed predominantly in recombinant cellular models and to a lesser extent within natural cellular environments (e.g., choroid plexus epithelial cells). Multiple G proteins (e.g., $G\alpha_{q/11}$, $G\alpha_{12/13}$, and $G\alpha_{i/o}$) and activation of second messengers such as phospholipases, cyclic nucleotides, and $ERK_{1/2}$ are essential mediators of 5-HT_{2C} receptor actions in cells. By the late 1990s, structurally diverse 5-HT_{2C} receptor agonists (Berg et al., 1994, 1998a; Moya et al., 2007) were noted to differentially activate intracellular signaling pathways, variably referred to as "agonist-directed trafficking of receptor stimulus," biased agonism (signaling), stimulus trafficking, collateral efficacy, and functional selectivity (Berg and Clarke, 2009; Whalen et al., 2011; Kenakin and Christopoulos, 2013). Recent modeling studies suggest that 5-HT_{2C} receptor ligands with fewer docking poses may stabilize a structural conformation contributory to a specific signaling pathway (Canal et al., 2011). Thus, distinct conformational states may differentially modulate the receptor interaction with immediate effectors (e.g., G proteins vs. β-arrestins), resulting in biased intracellular signal transduction patterns depending on the recruited effector (Gesty-Palmer et al., 2006; Whalen et al., 2011; Kenakin and Christopoulos, 2013). For example, G protein–dependent signaling pathways result in activation of specific downstream signaling effectors (e.g., $pERK_{1/2}$), whereas β-$arrestin_2$–dependent signaling can result in a different subset of downstream effectors as well as subcellular distribution of shared effectors (e.g., cytoplasmic vs. nuclear $pERK_{1/2}$). These signaling profiles can then lead to distinct overall effects of GPCR activation (Gesty-Palmer et al., 2006). Targeting G protein– versus β-$arrestin_2$–dependent mechanisms can allow for selectively inducing certain outcomes of receptor activation and perhaps not only reducing undesired side effects but also providing new therapeutic possibilities (Luttrell et al., 2015), a hypothesis that is supported for the 5-HT_{2C} receptor system (Berg and Clarke, 2009) as well as for other GPCR signaling systems (Gesty-Palmer et al., 2006; Masri et al., 2008; Allen et al., 2011; Lovell et al., 2015; Marti-Solano et al., 2015).

1. Phospholipase C. The 5-HT_{2C} receptor is characterized to stimulate phospholipase C (PLC) signaling through pharmacological analyses in heterologous expression systems (Westphal and Sanders-Bush, 1996; Briddon et al., 1998; Herrick-Davis et al., 1999; Rosendorff et al., 2000; Devlin et al., 2004), choroid plexus cells (Conn and Sanders-Bush, 1986b; Conn et al., 1986; Sanders-Bush and Conn, 1986), and corticostriatal regions of the brain (Wolf and Schutz, 1997). In this regard, the 5-HT_{2C} receptor is largely thought to act through pertussis toxin–insensitive $G\alpha_{q/11}$ proteins (Conn et al., 1986; Berg et al., 1994; Chang et al., 2000), although there is evidence that pertussis toxin–sensitive $G\alpha_{i/o}$ G proteins may couple the 5-HT_{2C} receptor to PLC in *Xenopus laevis* oocytes (Chen et al., 1994) and HEK293 cells (Alberts et al., 1999). Thus, the canonical G protein–dependent signaling through the 5-HT_{2C} receptor is engendered by 5-HT–stimulated coupling to $G\alpha_{q/11}$ to activate the enzyme phospholipase C_β(PLC_β), which generates the intracellular second messenger inositol-1,4,5-trisphosphate (IP_3), accumulation of the downstream IP_3 metabolite inositol monophosphate (IP_1), and DAG. IP_3 interacts with the IP_3 receptor, leading to increased i Ca_i^{2+} into the cytoplasm; Ca_i^{2+} mobilization, measured with calcium-binding fluorescent dyes, and IP_1 levels, assessed with [^{3}H]-inositol, are well characterized to be elevated following activation of the 5-HT_{2C} receptor [for reviews, see Raymond et al. (2001) and Millan et al. (2008)]. Utilizing cell-permeable small peptide disruptors mimicking the C terminal of $G\alpha_q$, Sanders-Bush and colleagues demonstrated that a $G\alpha_q$, but not a G_s, disruptor was able to block 5-HT_{2C} receptor–mediated phosphoinositide hydrolysis in choroid plexus endothelial cells (Chang et al., 2000). Furthermore, a $PLC_{\beta1}$, but not a $PLC_{\beta2}$, peptide blocked 5-HT_{2C} receptor activation, suggesting that 5-HT_{2C} receptor–evoked phosphoinositide hydrolysis

is mediated through a $G\alpha_q$- and $PLC_{\beta 1}$-dependent mechanism (Chang et al., 2000).

Hydrolysis of phosphoinositides generates the signaling lipid DAG, leading to activation of PKC and downstream stimulation of the MAPK cascade, resulting in phosphorylation of $ERK_{1/2}$ (Werry et al., 2005). In fact, 5-HT_{2C} receptor–transfected CHO cells were shown to couple $ERK_{1/2}$ via a PLD- and PKC-dependent pathway likely through $G\alpha_{12/13}$ proteins (Werry et al., 2005). The PLD and PKC involvement in $ERK_{1/2}$ phosphorylation evoked by 5-HT_{2C} receptor stimulation was recently validated in a hypothalamic cell line (mHypoA-2/10) derived from the periventricular nucleus of an adult male mouse (Lauffer et al., 2016). These further analyses indicated that the native 5-HT_{2C} receptor activates the cellular transcription factor CREB via PKC-induced $ERK_{1/2}$ activation in this cellular model (Lauffer et al., 2016).

Desensitization and resensitization processes regulate the functional activity of 5-HT_{2C} receptor. Agonist-dependent desensitization is associated with 5-HT_{2C} receptor phosphorylation involving G protein–coupled receptor kinase (GRK) 2 (Berg et al., 2001), binding of β-arrestins, and uncoupling of the receptor from the G protein to result in receptor internalization into endosomes; resensitization and recycling to the plasma membrane occurs with dephosphorylation (Marion et al., 2004; Schlag et al., 2004). The interaction of the 5-HT_{2C} receptor with the C-terminal domain of PSD-95/Disc large/Zonula occludens (PDZ) domain–containing proteins (Bécamel et al., 2002, 2004; Anastasio et al., 2010; Anastasio et al., 2014b) is known to play an important role in 5-HT_{2C} receptor desensitization/resensitization processes and trafficking (Gavarini et al., 2006). Intracellular Ca_i^{2+} release in mouse cortical neurons in primary culture is regulated by the PDZ proteins postsynaptic density 95 (PSD-95) and MAGUK p55 subfamily member 3 (MPP3) (Gavarini et al., 2006; Møller et al., 2013). Although PSD-95 and MPP3 do not modify the efficacy of 5-HT_{2C} receptor signaling triggered by a single 5-HT exposure, PSD-95 increases signal desensitization and trafficking upon repeated agonist exposure, an effect that is blocked by a peptidyl mimetic of the 5-HT_{2C} receptor C-terminus, which disrupts the interaction between the 5-HT_{2C} receptor and PSD-95 (Gavarini et al., 2006). On the other hand, MPP3 stabilizes the 5-HT_{2C} receptor at the plasma membrane and prevents desensitization of the 5-HT_{2C} receptor–mediated Ca_i^{2+} release (Gavarini et al., 2006). This regulation correlates with surface expression of the receptor and indicates that 5-HT_{2C} receptor signaling is highly regulated by PDZ proteins.

2. Phospholipase D. The 5-HT_{2C} receptor also activates PLD, an enzyme that catalyzes the conversion of phosphatidylcholine to choline and phosphatidic acid; phosphatidic acid transduces most of PLD-evoked activity, whereas soluble choline diffuses into the cytosol, but has little second messenger activity [for reviews, see Frohman (2015) and Nelson and Frohman (2015)]. The activation of PLD can occur through the canonical receptor/G protein/effector signal transduction cascade via $G\alpha_{12/13}$. In rat choroid plexus epithelial cells, 5-HT–evoked PLD activation occurs at levels similar to PLC activation; however, PLD activation is not downstream to G protein–linked PLC activation (McGrew et al., 2002). The 5-HT_{2C} receptor antagonist SB206553 and a peptide targeting the $G\alpha_{13}$, but not the $G\alpha_q$, subunit blocked 5-HT–evoked PLD activation (McGrew et al., 2002). Inactivation of RhoA GTPase in NIH3T3 cells stably expressing the 5-HT_{2C} receptor by the C3 exoenzyme from *Clostridia botulinum* blocks 5-HT_{2C} receptor–evoked PLD, but not PLC, signaling (McGrew et al., 2002). Also, in a NIH3T3 cell line derived from $G\alpha_{q/11}$-deficient mice, 5-HT_{2C} receptor–dependent stress fiber formation is dependent on $G\alpha_{13}$ and Rho signaling (Gohla et al., 1999). The PLD signaling was not seen in cells transfected with the 5-$HT_{2C\text{-}VGV}$ receptor; this highly edited isoform has a diminished ability to couple to $G\alpha_{13}$ and is unable to promote Rho GTPase activity (McGrew et al., 2004). Together, these studies suggest that 5-HT_{2C} receptor–mediated PLD signaling is dependent on $G\alpha_{13}$ activation of Rho, a property of this receptor that is affected by pre-RNA editing.

3. Phospholipase A_2. Stimulation of the 5-HT_{2C} receptor is thought to activate cytosolic PLA_2, which hydrolyzes arachidonic acid–containing phospholipids to produce free arachidonic acid and a host of its metabolites (for reviews, see Burke and Dennis, 2009a,b). In an early study, 5-HT was demonstrated to stimulate the release of arachidonic acid through activation of PLA_2, but not phosphoinositide turnover, in hippocampal neurons cocultured with glial cells, but not in glial cultures alone; the studies supported the involvement of the 5-HT_2 receptor subtype in the PLA_2 activation (Felder et al., 1990). In 5-HT_{2C} receptor–transfected CHO cells, 5-HT increases the release of arachidonic acid, an effect that is blocked by the PLA_2 inhibitor mepacrine, which had no effect on 5-HT_{2C} receptor–mediated phosphoinositide hydrolysis (Berg et al., 1996). The G protein effector is sensitive to pertussis toxin inactivation, but the exact G proteins involved are as of yet unknown (Felder et al., 1990). This research was extended to demonstrate that the relative efficacy of agonists is distinct for the PLA_2–arachidonic acid versus PLC-phosphoinositide pathways. For example, DOI acts as a full agonist to stimulate arachidonic acid release (equivalent to 5-HT), whereas 3-trifluoromethylphenylpiperazine and *d*-LSD preferentially activate the PLC-phosphoinositide and PLA_2–arachidonic acid pathways, respectively (Berg et al., 1998b). In addition, arachidonic acid release, though sensitive to pretreatment with 5-HT, is not as sensitive

as phosphoinositide hydrolysis, suggesting that arachidonic acid release may be more difficult to demonstrate in vivo as a 5-HT_{2C} receptor–mediated output when compared with phosphoinositide hydrolysis (Berg et al., 1998b). The involvement of arachidonic acid stimulation in 5-HT_2 receptor signaling in vivo is supported by the observation that systemic administration of DOI resulted in increased incorporation of labeled arachidonic acid into brain membranes (Qu et al., 2005). The increases were seen in brain regions with the highest densities of 5-HT_{2A} receptor (e.g., cerebral cortex) but not in the choroid plexus, which expresses the highest 5-HT_{2C} receptor density (choroid plexus). Future studies are necessary to clarify the relative roles of the 5-HT_{2A} receptor and 5-HT_{2C} receptor in the control of arachidonic acid incorporation in vivo (Basselin et al., 2012).

4. Cyclic Nucleotides. Signaling through cyclic nucleotides, such as cAMP and cGMP, are also reported to be engaged by the 5-HT_{2C} receptor. Activation of the 5-HT_{2C} receptor inhibits forskolin-stimulated cAMP production in AV12 fibroblast cells that stably express the receptor at high density (~12 pmol/mg membrane protein); at low 5-HT_{2C} receptor density (~150 fmol/mg of membrane protein), 5-HT couples to the PLC-IP pathway but evokes a stimulation rather than an inhibition of cAMP production (Lucaites et al., 1996). Upon pertussis toxin treatment, a modest 5-HT_{2C} receptor stimulatory effect on cAMP accumulation as well as its potential dependence on the $G_{i/o}$ family of G proteins was observed (Lucaites et al., 1996). In *Xenopus* oocytes transfected with the 5-HT_{2C} receptor and G proteins, the 5-HT_{2C} receptor was shown to couple to G_o in addition to G_q (Quick et al., 1994). Because there is limited evidence that the 5-HT_{2C} receptor links to cAMP in cells natively expressing the receptor (porcine choroid plexus) (Palacios et al., 1986), further analyses as to its biologic significance in vitro and in vivo are required.

The antimigraine medication dihydroergotamine, which inhibits [^{3}H]-mesulergine binding to the 5-HT_{2C} receptor and is a full 5-HT_{2C} receptor agonist in porcine choroid plexus (Brown et al., 1991), elevates cGMP levels in $LMTK^-$ fibroblasts stably expressing the 5-HT_{2C} receptor (Schaerlinger et al., 2003). 5-HT rapidly elevates cGMP production in the 5-HT_{2C} receptor–enriched porcine choroid plexus tissue slices (Kaufman et al., 1995) with an efficacy similar to phosphatidyl inositol turnover (Conn et al., 1986); the potencies of antagonists that suppress 5-HT–mediated cGMP formation align with their affinity for the 5-HT_{2C} receptor (Kaufman et al., 1995). The pertussis toxin–insensitive 5-HT_{2C} receptor–mediated cGMP formation is dependent on calcium and PLA_2-arachidonate release as well as lipoxygenase (Kaufman et al., 1995). Thus, the 5-HT_{2C} receptor appears to exhibit efficacy to evoke cGMP formation in a native tissue in a PLA_2-dependent manner (Kaufman et al., 1995) as well as regulate NMDA-mediated production of nitric oxide and elevation of cGMP (Marcoli et al., 1997).

5. Extracellular Signal-Regulated Kinases. The activation of the 5-HT_{2C} receptor can diverge to influence several G protein–dependent downstream cascades and may also converge on others, such as members of the MAPK class, P42 and 44 (p44/p42-MAPK), also known as $ERK_{1/2}$. In fact, there is evidence to suggest that phosphorylation of $ERK_{1/2}$ is an important integrator of the multiple upstream signaling events for the 5-HT_{2C} receptor. In CHO cells stably expressing the 5-HT_{2C} receptor, 5-HT stimulated phosphorylation of $ERK_{1/2}$, which is inhibited by the 5-HT_{2C} receptor antagonist mianserin (Werry et al., 2005). Though PKC and PLD inhibitors suppress 5-HT–mediated phosphorylation of $ERK_{1/2}$, PLC and PLA_2 inhibitors are ineffective, suggesting the 5-HT_{2C} receptor–mediated p$ERK_{1/2}$ is dependent on PKC and PLD signaling (Werry et al., 2005). Activation of $ERK_{1/2}$ by 5-HT_{2C} receptor ligands is not solely mediated by coupling of the 5-HT_{2C} receptor to G proteins and can, in fact, be mediated by coupling to other protein transducers. The C terminus of the 5-HT_{2C} receptor contains a calmodulin domain that is critical for β-arrestin recruitment and $ERK_{1/2}$ signaling (Labasque et al., 2008). A calmodulin mutant prevented phosphorylation of $ERK_{1/2}$ in cortical neurons and choroid plexus epithelial cells, suggesting that the calmodulin interaction is critical for G protein–independent signaling (Labasque et al., 2008). In HEK293 cells transiently expressing the 5-HT_{2C} receptor, p$ERK_{1/2}$ levels were increased over nontransfected cells, suggesting the expression of constitutive activity of the 5-HT_{2C} receptor (Labasque et al., 2008). This elevated basal p$ERK_{1/2}$ was inhibited by a 5-HT_{2C} receptor inverse agonist toward PLC (Labasque et al., 2008), again suggesting that p$ERK_{1/2}$ activity is PLC-mediated. Interestingly, this activity was unaffected by depletion of $G\alpha_{12}$ or $G\alpha_{13}$ proteins, whereas in cells lacking β-arrestin or calmodulin, basal p$ERK_{1/2}$ levels were decreased, providing the first evidence of constitutive activity of a G protein–coupled receptor toward a G protein–independent, β-arrestin–dependent signaling mechanism (Labasque et al., 2008).

6. Ion Channels. The impact of 5-HT_{2C} receptor on the function of ion channels has been addressed extensively in the choroid plexus, which controls the composition and secretion of cerebrospinal fluid, a function in which chloride (Cl^-) and potassium (K^+) channels play a key role (Millar et al., 2007). 5-HT acting through the 5-HT_{2C} receptor has been noted to activate Cl^- and inhibit K^+ channels in mouse and rat choroid plexus epithelium (Hung et al., 1993; Speake et al., 2004), and the 5-HT_{2C} receptor has been shown to inhibit K^+ channels through a PKC-dependent pathway in rat choroid plexus epithelial cells (Speake et al., 2004). Activation of the 5-HT_{2C} receptor leads to G protein–dependent PLC activation, IP_3 and DAG production, and

the release of Ca_i^{2+} from the endoplasmic reticulum, which triggers the opening of calcium-activated Cl^- channels in *Xenopus* oocytes (Julius et al., 1988; Panicker et al., 1991; Woodward et al., 1992; DiMagno et al., 1996). The G_α and G_q subunits may be involved in the generation of this calcium-activated Cl^- current, which also involves PLCβ production (Quick et al., 1994; DiMagno et al., 1996). Upon coexpression of the 5-HT_{2C} receptor and a brain-derived K^+ channel in *Xenopus* oocytes, 5-HT suppresses K^+ conductance through a calcium/calmodulin-activated phosphatase thought to dephosphorylate the K^+ channel in a kinase-dependent manner, evoking its closure (Hoger et al., 1991).

The 5-HT_{2C} receptor also modulates the Kv1.5 channel through a PLC-dependent pathway in *Xenopus* oocytes (Panicker et al., 1991), which contrasts the observation that the 5-HT_{2C} receptor inhibits K^+ channels through a PKC-dependent pathway in rat choroid plexus epithelial cells (Speake et al., 2004). The 5-HT_{2C} receptor also inhibits the $GABA_A$ receptor in *Xenopus* oocytes through a calcium-dependent, phosphorylation-independent mechanism (Huidobro-Toro et al., 1996b) and by suppressing an inwardly rectifying K^+ current in striatal cholinergic interneurons and rat brain slices (Blomeley and Bracci, 2005; Blomeley and Bracci, 2009). The 5-HT_{2C} receptor was $G\alpha_q$-coupled to PLC activation and phosphoinositide hydrolysis to directly inhibit GIRK channels in POMC neurons of the arcuate (Qiu et al., 2007) as well as in lateral (but not basolateral) amygdala neurons (Yamamoto et al., 2014), which are known to stabilize resting membrane potential and therefore polarization of these neurons (Delmas and Brown, 2005). Hence, though stimulation of 5-HT_{2C} receptor significantly modulates ion channel function, the involvement of specific signal transduction molecules in the processes is variable and underexplored at present.

F. The 5-HT_{2C} Receptor as an Oncogene

5-HT is known to act as a mitogen to regulate the proliferation and differentiation of a variety of cells through its binding to several 5-HT receptors and control of downstream signaling (for review, see Fanburg and Lee, 1997). The NIH3T3 mouse fibroblast cells are often used to identify oncogenes (genes that can transform a cell to a tumor cell) because they display several well characterized phenotypes that are indicative of cellular transformation, including the formation of foci (regions of dense cell growth within an otherwise confluent monolayer) (Land et al., 1983). Stimulation of NIH3T3 cells transfected with the 5-HT_{2C} receptor leads to the generation of transformed foci, maintenance of which requires the activation of the 5-HT_{2C} receptor as a 5-HT_{2C} receptor antagonist blocks foci formation (Julius et al., 1988; Julius et al., 1989). Upon injection of these cells into nude mice, tumors form, a finding that led to the initial conclusion that the 5-HT_{2C} receptor is a proto-oncogene (Julius et al., 1989). The constitutive activity of the 5-HT_{2C} receptor also stimulated cell division in the transfected NIH3T3 cells, and these data further suggested that multiple G proteins and signaling pathways were engaged in the cell division generated by agonist-evoked and constitutively active receptor signaling (Westphal and Sanders-Bush, 1996).

G. Clinical Relevance of the 5-HT_{2C} Receptor

The 5-HT_{2C} receptor is widely distributed throughout the basal ganglia, limbic system, and prefrontal cortex (Hoyer et al., 1986; Hoffman and Mezey, 1989; Molineaux et al., 1989; Mengod et al., 1990) and is well poised to mediate 5-HT–dependent appetite, cognition, mood, movement, and sleep, whereas dysfunctional 5-HT_{2C} receptor signaling has been implicated in neuropsychiatric (e.g., addiction, anxiety, and depression) and neuropathological conditions (e.g., schizophrenia) as well as obesity and metabolic disorders (Berg et al., 2008a; Di Giovanni et al., 2010; Fig. 14). Therefore, the 5-HT_{2C} receptor is a therapeutic target of great interest (for other reviews, see Cunningham and Anastasio, 2014; Howell and Cunningham, 2015; Sullivan et al., 2015; Di Giovanni and De Deurwaerdere, 2016; Fig. 12). The behavioral pharmacology of 5-HT_{2C} receptor ligands as well as the clinical relevance of a dysfunctional 5-HT_{2C} receptor system has recently been reviewed in detail (Cunningham and Anastasio, 2014; Howell and Cunningham, 2015; Sullivan et al., 2015; Di Giovanni and De Deurwaerdere, 2016), and an overview of clinical implications of the 5-HT_{2C} receptor system is provided here.

1. Substance Use Disorders (Addiction). The pharmacological and molecular mechanisms underlying the effects of abused drugs have been the subject of extensive study. As early work unfolded, a central concept emerged that the dopamine pathway projection from the VTA to the nucleus accumbens plays a mechanistic role in the rewarding and incentive-salience value of abused drugs (for reviews, see Koob and Volkow, 2010; Volkow et al., 2010). As the research progressed, the field began to recognize that the transition to problematic drug abuse and substance use disorder (addiction and dependence) involves an "expanding cycle of dysfunction" (Koob and Volkow, 2010), engaging multiple neurotransmitter substrates within limbic-corticostriatal circuitry, including 5-HT (for reviews, see Kalivas and Volkow, 2005; Koob and Volkow, 2010; Volkow et al., 2010; Cunningham and Anastasio, 2014; Muller and Homberg, 2015; Di Giovanni and De Deurwaerdere, 2016; Wolf, 2016). Much of the research has focused on abused drugs as rewarding substances and the evoked, long-lasting dysregulation of the dopamine mesoaccumbens pathway (for reviews, see Koob and Volkow, 2010; Volkow et al., 2010). However, a second focus has evolved toward identifying genotypic and phenotypic drivers of individual differences in

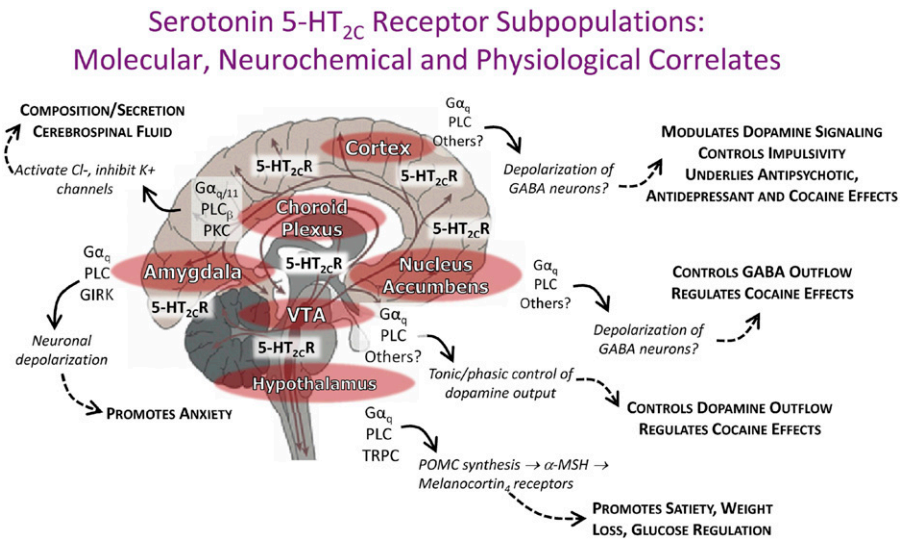

Fig. 14. Regional CNS localization 5-HT$_{2C}$ receptors and functional correlates. The full-length 5-HT$_{2C}$ receptor is localized exclusively in the central nervous system with good agreement between mRNA and protein distribution in the majority of brain regions. The postulated signaling components (shown in normal text), neurochemical and/or neurophysiological correlates (shown in italic text), and in vivo effects (shown in CAPITAL text) are illustrated.

vulnerability to substance use disorders and relapse, as drug abuse culminates in addiction in only a subset of users (SAMHSA, 2015). Impulsivity, a predisposition toward rapid unplanned reactions to stimuli without regard to the negative consequences, is one such phenotype that contributes to initial drug use and is perpetrated by continued use of the abused drug (for reviews, see Moeller et al., 2001a,b; Cunningham and Anastasio, 2014). The impact of impulsivity in psychostimulant addiction is best described with roles for 5-HT and dopamine prominently identified (for reviews, see Moeller et al., 2001a; Dalley and Roiser, 2012; Bari and Robbins, 2013; Cunningham and Anastasio, 2014; Logue and Gould, 2014). Cue reactivity, the sensitivity to cues previously linked with the drug-taking experience, is a second such phenotype that plays a prominent role in craving and relapse in humans (Carter and Tiffany, 1999; O'Brien et al., 1998; Drummond, 2001). The extended limbic-cortico-striatal circuitry underlies both impulsivity and cue reactivity with multiple neurotransmitters involved (Childress et al., 1999; Bechara, 2005; Goldstein et al., 2007; Liu et al., 2012). Thus, addiction involves the generation of drug use within a background of vulnerability (e.g., propensity for impulsive behavior) and progresses with repeated drug exposure, neuronal plasticity, and the entrainment of addictive behaviors. In particular, the role of the 5-HT$_{2C}$ receptor in various aspects of these addictive processes has been well studied for the class of psychostimulants; several studies have reported the efficacy of 5-HT$_{2C}$ receptor agonists to suppress nicotine intake and nicotine seeking (Grottick et al., 2001; Levin et al., 2011; Higgins et al., 2012). Relevant to this are the extensive studies of the role of the 5-HT$_{2C}$ receptor in the rewarding and incentive-salience value of cocaine as well as factors involved in vulnerability to addiction and relapse, especially impulsivity and cue reactivity.

Cocaine is a psychomotor stimulant that inhibits 5-HT reuptake (Koe, 1976). Employing the self-administration assay, the preclinical model with the best validity for human drug taking, studies demonstrated that voluntary cocaine administration elevates 5-HT efflux in the nucleus accumbens (Parsons and Justice, 1993; Parsons et al., 1996; Howes et al., 2000). Depletion of forebrain 5-HT induces compulsive cocaine seeking, which is reversed by a 5-HT$_{2C}$ receptor antagonist (Pelloux et al., 2012). Constitutive knockout of the 5-HT$_{2C}$ receptor increases the motivation to take cocaine and enhances cocaine-induced elevation in dopamine in the nucleus accumbens (but not the dorsal striatum) of mice (Rocha et al., 2002). Pretreatment with a 5-HT$_{2C}$ receptor agonist, systemically or into the VTA, enhances, whereas systemic administration of a 5-HT$_{2C}$ receptor antagonist inhibits, the elevated dopamine efflux in the NAc evoked by nonresponse contingent cocaine administration (Navailles et al., 2004, 2008; Cathala et al., 2015). The 5-HT$_{2C}$ receptor control over dopamine function is thought to mediate, in large part, the efficacy of a selective 5-HT$_{2C}$ receptor agonist (e.g., RO60-0175 or WAY163909) to suppress the voluntary intake of cocaine (Grottick et al., 2000; Fletcher et al., 2002a; Fletcher et al., 2004; Neisewander and Acosta, 2007; Cunningham et al., 2011).

Stimulation of the 5-HT$_{2C}$ receptor also dose-dependently suppresses reinstatement induced by cocaine and cocaine-associated cues as measures of cue reactivity (Grottick et al., 2000; Neisewander and Acosta, 2007; Burbassi and Cervo, 2008; Fletcher et al., 2008; Cunningham et al., 2011; Swinford-Jackson et al., 2016). Conversely, systemic administration of 5-HT$_{2C}$

receptor antagonists have been shown to exert effects opposite to those following agonist administration, thus enhancing cocaine self-administration (Fletcher et al., 2002a) and cue reactivity (Fletcher et al., 2002a; Pelloux et al., 2012). In nonhuman primates, a 5-HT_{2C} receptor agonist attenuated the stimulant, reinforcing, and reinstatement effects of cocaine, effects reversed by the selective 5-HT_{2C} receptor antagonist SB242084 (Manvich et al., 2012a,b; Ruedi-Bettschen et al., 2015). Interestingly, SB242084 induced modest stimulant effects and exhibited reinforcing effects in primates (Manvich et al., 2012a,b) but had contrasting results (Ruedi-Bettschen et al., 2015).

The efficacy of 5-HT_{2C} receptor agonists to suppress cue reactivity upon systemic administration is mirrored following intracranial microinjection of a 5-HT_{2C} receptor agonist into the mPFC, which attenuates both cocaine- and cue-induced reinstatement (Pentkowski et al., 2010). These data highlight the importance of the 5-HT_{2C} receptor in regulation of cortical substrates underlying cocaine-associated cue reactivity specifically, as 5-HT_{2C} receptor agonist microinfusions did not alter cocaine intake (Pentkowski et al., 2010). Thus, the 5-HT_{2C} receptor provides inhibitory tone over cocaine reward and cue reactivity as well as the neurochemical effects of cocaine (for reviews, see Cunningham and Anastasio, 2014; Howell and Cunningham, 2015; Di Giovanni and De Deurwaerdere, 2016), and mPFC-localized 5-HT_{2C} receptors underlie, in part, the generation of cue reactivity.

Cocaine administered nonresponse contingently has been reported to result in 5-HT_{2C} receptor neuroadaptations (Zayara et al., 2011; Craige et al., 2015) as well as regulation of the brain-specific snoRNA MBII-52, which is involved in the regulation of the 5-HT_{2C} receptor pre-mRNA (Chen et al., 2014). Abstinent cocaine users exhibit lower sensitivity to the effects of a 5-HT_{2C} receptor agonist (Lee and Meltzer, 1994; Buydens-Branchey et al., 1997; Patkar et al., 2006), whereas the highest cue reactivity was observed in those cocaine-dependent subjects carrying the C23S SNP in the 5-HT_{2C} receptor gene (Anastasio et al., 2014a), which may be associated with diminished 5-HT_{2C} receptor signal transduction (Lappalainen et al., 1995; Okada et al., 2004; Piva et al., 2011; Walstab et al., 2011). Further evidence that the functional status of the 5-HT_{2C} receptor in the mPFC (Lopez-Gimenez et al., 2001; Liu et al., 2007; Nocjar et al., 2015) influences the incentive-motivational effects of cocaine and cocaine-associated cues has accumulated in rats (Anastasio et al., 2014a,b; Swinford-Jackson et al., 2016). Cunningham and colleagues found that the highest levels of cocaine cue reactivity correlated with the lowest levels of mPFC 5-HT_{2C} receptor protein and a blunted sensitivity to the suppressive effects of the selective 5-HT_{2C} receptor agonist WAY163909 (Anastasio et al., 2014a; Swinford-Jackson et al., 2016). The efficacy of WAY163909 to suppress high levels of cue reactivity associated with extended forced abstinence ("incubation") was also reduced at a time point (30 days) at which lower synaptosomal expression of 5-HT_{2C} receptor protein was observed in the mPFC (Swinford-Jackson et al., 2016), a key site involved in incubation phenomena (Koya et al., 2009; Whitfield et al., 2011; Ma et al., 2014). Furthermore, a greater proportion of the expressed 5-HT_{2C} receptor protein was sequestered in the cytoplasmic (vs. membrane) compartment of the mPFC at prolonged versus early forced abstinence, and there was an inverse correlation of the membrane to cytoplasmic 5-HT_{2C} receptor ratio in the mPFC with levels of cocaine cue reactivity. Collectively, these outcomes indicate that the functional status of the 5-HT_{2C} receptor system in the mPFC is a key contributor to cocaine cue reactivity and its incubation.

Cocaine-dependent subjects who express high cocaine cue reactivity express high impulsivity (Liu et al., 2011b), and a similar relationship has been observed in cigarette smokers (Doran et al., 2007, 2008). In outbred rats, lower mPFC 5-HT_{2C} receptor membrane protein levels and an increase in edited 5-HT_{2C} receptor mRNA variants with reduced 5-HT_{2C} receptor signaling capacity distinguish high impulsive rats from low impulsive rats (Anastasio et al., 2014b) as well high and low responders to novelty, another model of addiction vulnerability (Dracheva et al., 2009). The virally mediated knockdown of the 5-HT_{2C} receptor localized to the mPFC also results in elevated impulsivity and cue reactivity relative to controls (Anastasio et al., 2014b), suggesting that reduced 5-HT_{2C} receptor tone in the mPFC confers vulnerability to these interlocked behaviors (Anastasio et al., 2014b). The status of 5-HT_{2C} receptor function in the orbitofrontal cortex may also be a contributor to the vulnerability of impulsive rats to cocaine reward and cue reactivity (Besson et al., 2013). Together, these data suggest that the functional status of the cortical 5-HT_{2C} receptor system may be a mechanistic driver in the generation of cocaine use disorder and relapse phenomena.

The body of knowledge in support of a role of the 5-HT_{2C} receptor in regulating the rewarding properties and voluntary intake of other abused substances is less well developed than that of cocaine and nicotine. However, stimulation of the 5-HT_{2C} receptor has been noted to suppress ethanol self-administration (Maurel et al., 1999; Tomkins et al., 2002; Kasper et al., 2013; Rezvani et al., 2014) and reinstatement in rodents (Kasper et al., 2013). Exposure to ethanol vapor for several days is associated with increased expression of the 5-HT_{2C} receptor transcript in several corticostriatal and hypothalamic nodes (Yoshimoto et al., 2012), higher levels of the 5-HT_{2C} receptor protein in the NAc (Yoshimoto et al., 2012), and enhanced pre-mRNA editing of the 5-HT_{2C} receptor (Watanabe et al., 2014), suggesting that neuroadaptations in the 5-HT_{2C}

receptor function are mechanistically involved in ethanol preference behavior.

Pretreatment with 5-HT_{2C} receptor ligands have been shown to impact various behavioral sequela associated with *d*-amphetamine (O'Neill et al., 1999; Rippberger et al., 2015; Wöhr et al., 2015), MDMA (Bankson and Cunningham, 2002; Fletcher et al., 2002b), methamphetamine (Steed et al., 2011; Graves and Napier, 2012), and the marijuana alkaloid Δ^9-THC (Ji et al., 2006), suggesting that rich prospects to explore the potential therapeutic value of selective 5-HT_{2C} receptor agonists in addictive processes engaged by these abused drugs remain.

5-HT is involved in the pharmacology of opioid abused drugs (including heroin and prescription opioids) (Tao and Auerbach, 1994; Tao et al., 1998; Singh et al., 2003), and systemic administration of a 5-HT releaser (e.g., dexfenfluramine) was shown to suppress heroin self-administration in rats (Wang et al., 1995). Pretreatment with a selective 5-HT_{2C} receptor agonist also reduced opioid-induced behavioral sensitization (Wu et al., 2015a; Zhang et al., 2016). Recent studies have further demonstrated that lorcaserin significantly decreases the reinforcing effects of oxycodone and shifts the oxycodone dose-effect curve downward at doses that do not alter motor activity (Neelakantan et al., 2017). Thus, 5-HT_{2C} receptor agonists may prove therapeutically useful to promote recovery and extend abstinence from several classes of abused drugs.

2. Appetite, Satiety, and Obesity. Hunger is the physiologic need for food. Appetite (the desire for food), satiation (the end of the desire for food during a meal), and satiety (the feeling of "fullness" that prevents further eating before the return of hunger) include both internal (e.g., glucose homeostasis) and conditioned factors (e.g., hedonics) (Blundell, 1999). 5-HT in the CNS has long been implicated in the control of the processes involved in satiation and satiety (Lucki, 1998; Halford and Blundell, 2000; Saper et al., 2002; Voigt and Fink, 2015). The 5-HT releaser *d*-fenfluramine, employed clinically for weight loss (until withdrawn in 1997), its metabolite *d*-norfenfluramine, and preferential 5-HT_{2C} receptor agonists (e.g., mCPP) evoke hypophagia in rodents, which is associated with increased satiety, an effect blocked by 5-HT_{2C} receptor antagonists or constitutive knockdown of the 5-HT_{2C} receptor (Kennett and Curzon, 1988; Tecott et al., 1995; Halford et al., 1997; Vickers et al., 1999; Dalton et al., 2006; Nonogaki et al., 2008). Selective 5-HT_{2C} receptor agonists (e.g., lorcaserin, RO60-0175) have been consistently demonstrated to suppress food intake (Clifton et al., 2000; Somerville et al., 2007; Thomsen et al., 2008; Grottick et al., 2015; Higgs et al., 2015; for review, see Higgins et al., 2020). In fact, the selective 5-HT_{2C} receptor agonist WAY163909 dose-dependently decreases food intake in normal Sprague-Dawley rats, obese Zuker rats, and mice with diet-induced obesity (Dunlop et al., 2005) without the anxiogenic profile of mCPP (Dunlop et al., 2006). Tecott et al. (1995) reported that the constitutive 5-HT_{2C} receptor knockout mouse exhibited hypophagia and increased body mass in the context of both insulin resistance and late-onset obesity (Nonogaki et al., 2008), whereas weight gain as well as a greater relative risk of metabolic dysfunction and diabetes develops with the chronic treatment of atypical antipsychotics with 5-HT_{2C} receptor antagonist properties (e.g., olanzapine) in humans and animals (Wirshing et al., 1999; Kirk et al., 2009). Interestingly, a selective 5-HT_{2C} receptor antagonist has been reported to variably increase (Bonhaus et al., 1997) or decrease food intake depending on the preclinical model employed (Kennett et al., 1997; Murotani et al., 2011).

The behavioral satiety sequence (BSS) describes the orderly process through which eating transitions to other behaviors (e.g., grooming and resting) and is a well validated model for analyzing satiation (meal termination) and satiety (postingestive inhibition of food intake) in rodents and humans (for review, see Rodgers et al., 2010). Consistent with the proposed role of 5-HT to promote satiety, *d*-fenfluramine, its metabolite analogs, and preferential and selective 5-HT_{2C} receptor agonists (Halford et al., 1998; Clifton et al., 2000; Hewitt et al., 2002; Dalton et al., 2006; Somerville et al., 2007) accelerate the BSS without disruption of its integrity (for review, see Rodgers et al., 2010). For example, *d*-fenfluramine and RO60-0175 reduced the rate of feeding and meal size as well as increased the latency to feed consistent with enhanced satiety (Clifton et al., 2000). The effects of *d*-fenfluramine on the BSS was markedly reduced in mice constitutively lacking the 5-HT_{2C} receptor (Vickers et al., 1999). In contrast, appetite-enhancing drugs (e.g., cyproheptadine) (Chinuck et al., 2007) disrupt the satiety sequence (Bergen, 1964; Ishii et al., 2003). A recent microstructural analysis of ingestive behavior found that lorcaserin reduced the number of bouts of licking behavior (Higgs et al., 2016) indicative of the promotion of satiety (Davis et al., 2001). Thus, the 5-HT_{2C} receptor is an important mediator of food intake through the control of satiety mechanisms (for reviews, see Lucki, 1998; Halford and Blundell, 2000; Voigt and Fink, 2015).

Investigations of 5-HT involvement in the mechanisms underlying satiety have focused predominantly on neural loci in the hypothalamus and midbrain/hindbrain circuits, which synchronize energy balance and glucose homeostasis in concert with peripheral systems (for reviews, see Saper et al., 2002; Gautron et al., 2015; Voigt and Fink, 2015). The 5-HT neurons in the dorsal and median raphe innervate multiple hypothalamic subnuclei (van de Kar and Lorens, 1979; Peyron et al., 1998), which richly express 5-HT_{2C} receptor mRNA and protein (Hoffman and Mezey, 1989; Molineaux et al., 1989; Mengod et al., 1990). A subpopulation of arcuate POMC neurons

express the 5-HT_{2C} receptor and are activated by *d*-fenfluramine and mCPP (Heisler et al., 2002; Lam et al., 2008). Elmquist and colleagues elegantly demonstrated that the activation of the 5-HT_{2C} receptor localized to POMC neurons stimulates POMC synthesis and its cleavage into α-melanocyte–stimulating hormone, which acts on melanocortin 4 receptors in the paraventricular nucleus of the hypothalamus to promote satiety, weight loss, and glucose regulation (Heisler et al., 2007a; Zhou et al., 2007; Xu et al., 2008; Berglund et al., 2013). Stimulation of the 5-HT_{2C} receptor following application of mCPP depolarized a subpopulation of POMC neurons potentially via PLC-dependent activation of transient receptor potential channels, independent of GIRK channel activity (Sohn et al., 2011). In mice that selectively lack the 5-HT_{2C} receptor in POMC neurons, body weight was normal; however, these mice were insensitive to *d*-fenfluramine– or mCPP-evoked hypophagia and developed metabolic dysfunction, including hyperinsulinemia, hyperglucagonemia, hyperglycemia, and insulin resistance (Berglund et al., 2013). Rescue of 5-HT_{2C} receptor in POMC neurons of 5-HT_{2C} receptor–null mice normalized food intake, adiposity, and body weight as well as the anorexigenic effects of *d*-fenfluramine and mCPP (Xu et al., 2008). Interestingly, POMC expression within 5-HT_{2C} receptor–expressing neurons in the arcuate regulates whole body energy balance, body weight, and adiposity in male, but not female, mice; these authors proposed that this molecular mechanism may explain, in part, sex differences in the prevalence of obesity (Burke et al., 2016). This provocative idea requires further investigation; however, the observations corroborate the conclusion that the 5-HT_{2C} receptor in POMC neurons is a required component of the neural mechanisms that control energy and glucose homeostasis.

The adipocyte-derived *ob* gene product leptin (Halaas et al., 1995) acts via the leptin receptor (Tartaglia et al., 1995) to regulate long-term energy stores and homeostasis (Friedman, 2014). This landmark discovery marshalled in an era of advanced understanding of the CNS circuits involved in obesity [for review, see Friedman (2014)]. It was not long before genetic deletion of the leptin receptor in neurons was shown to result in hyperphagia and obesity (Cohen et al., 2001), whereas the rescue of central leptin receptor signaling corrects the obesity and diabetes phenotype of *db*/*db* mice, which possess mutations in the leptin receptor (de Luca et al., 2005). Mice deficient for both leptin and the 5-HT_{2C} receptor exhibited a synergistic disruption of glucose homeostasis and a profound diabetes phenotype (Wade et al., 2008). On the other hand, transgenic mice that overexpress leptin but lack the 5-HT_{2C} receptor exhibited a lean phenotype on a chow diet; however, on a high-fat diet, these mice developed diet-induced obesity (Wang and Chehab, 2006). In POMC neurons, pharmacological stimulation of the 5-HT_{2C} receptor was shown to activate the same POMC neurons activated by leptin (Qiu et al., 2010). Lastly, coadministration of the preferential 5-HT_{2C} receptor agonist mCPP plus leptin was recently shown to have an additive effect on reducing body weight in diet-induced obese mice (Yan et al., 2015). The growing knowledge of the interface between leptin and 5-HT regulatory systems prompted Halford and Blundell to propose that these systems function independently, but coordinate within the hypothalamus, to control satiety and energy reserves (Halford and Blundell, 2000).

The drive to eat palatable foods, composed essentially of high fat and/or sugars, involves hedonic mechanisms, which are also important for maintaining the homeostatic nutritional requirements for energy balance [for reviews, see Saper et al. (2002) and Volkow et al. (2012)]. These foods activate limbic-corticostriatal systems, which mediate reward and motivation [for reviews, see Gautron et al. (2015) and Voigt and Fink (2015)], and hedonic eating has been identified is a contributor to the obesity epidemic (Saper et al., 2002; Volkow et al., 2012). Preclinical analysis of the rewarding (reinforcing) and motivational effects of food self-administration can be evaluated on a fixed ratio and a progressive ratio (PR) schedule, respectively, in either freely fed or food-restricted animals. Performance under PR schedules is thought to reflect the motivational "efficacy" of the food given that deprivation level as well as reinforcer magnitude can increase breakpoints on the PR schedule (Hodos and Kalman, 1963). The preferential (mCPP, MK212) and selective 5-HT_{2C} receptor agonists (R0 60-0175) decrease the breakpoint for a grain reinforcer on a PR schedule in food-restricted pigeons (Wolff and Leander, 2000). WAY163909 dose-dependently reduces self-administration of sucrose on a fixed ratio schedule in freely fed rats, an effect blocked by pretreatment with SB242084 (Cunningham et al., 2011), whereas mCPP decreases the breakpoint for Ensure in freely fed mice (Ward et al., 2008). Lorcaserin efficaciously suppresses the rewarding effects of food in food-restricted rats based on its 5-HT_{2C} receptor agonist actions [Higgins et al., 2012; for review, see Higgins et al. (2020)]. Interestingly, though 5-HT_{2C} receptor agonists effectively suppress cocaine seeking [for reviews, see Cunningham and Anastasio (2014) and Howell and Cunningham (2015)], WAY163909 failed to affect sucrose seeking (Cunningham et al., 2011), suggesting that the 5-HT_{2C} receptor differentially regulates the incentive-salience value of cocaine- versus sucrose-associated cues. Thus, there is evidence that the 5-HT_{2C} receptor system controls the hedonic, rewarding aspects of palatable food; the brain locus of action for the 5-HT_{2C} receptor to control these behaviors requires further evaluation (Pratt et al., 2009; Pratt et al., 2012; Clissold et al., 2013).

Multiple lines of investigation suggest a relationship between 5-HT_{2C} receptor SNPs and obesity,

antipsychotic-induced weight gain, and transcriptional activity of the *HTR2C* gene. Eight *HTR2C* gene polymorphisms have been reported in the literature: three polymorphisms, as well as a GT nucleotide repeat variation, have been identified in the promoter (Xie et al., 1996); three polymorphisms have been reported within intronic regions (Gibson et al., 2004); one polymorphism has been reported in the coding region, resulting in the replacement of cysteine with serine at amino acid 23 (C23S) in the amino-terminus of the receptor (Lappalainen et al., 1995); and there is one polymorphism in the 3′ untranslated region (Song et al., 1999).

The C23S SNP occurs with a frequency of approximately 10%–15% in the human population (Lappalainen et al., 1995). However, there is no evidence for an association of this SNP with obesity (Lentes et al., 1997; Gibson et al., 2004). The promoter haplotype −995A/−759T/−697C has been reported to be associated with obesity in a Japanese population (Yuan et al., 2000) and the −759C allele to be more common in obese than nonobese Caucasian women (Pooley et al., 2004). Several studies suggest that the −759T allele may be associated with less weight gain following antipsychotic drug treatment (Reynolds et al., 2003; Miller, 2005; Templeman et al., 2005). Although additional research has reported no association between antipsychotic-induced weight gain (AIWG) and *HTR2C* promoter polymorphisms (Basile et al., 2002; Tsai et al., 2002; Theisen et al., 2004; Templeman et al., 2005), others have reported an association of the −759C allele with AIWG (Wallace et al., 2011). It is interesting to note that greater promoter activity, resulting in increased *HTR2C* transcription, has been reported to be associated with the −759T allele (Yuan et al., 2000). Also, in a luciferase-based reporter assay, *HTR2C* promoter haplotypes containing the −759C allele showed lower transcriptional activity than those containing the −759T haplotype (Buckland et al., 2005). Although these studies suggest that the −759C/T polymorphism may regulate gene transcription in vitro, a subsequent study reported that 5-HT_{2C} receptor mRNA levels in the frontal cortex of 43 subjects are unaffected by −759C/T status (Pooley et al., 2004).

3. Schizophrenia. Both selective 5-HT_{2C} receptor agonists and 5-HT_{2C} receptor antagonists have been suggested for the treatment of schizophrenia. Antipsychotic medications with a profile as a 5-HT_{2C} receptor antagonist seem to be effective to suppress positive symptoms, whereas 5-HT_{2C} receptor agonists appear useful for inhibition of the negative symptoms and cognitive impairments in schizophrenia (Wood et al., 2001; Rosenzweig-Lipson et al., 2007a, 2012) with fewer motor side effects (Di Giovanni et al., 2006; Di Giovanni and De Deurwaerdere, 2016). The therapeutic potential of a specific antipsychotic would depend indirectly on the opposite modulation that these receptors exert on dopaminergic systems and the preferential 5-HT_{2C} receptor modulation of the mesocortical and limbic system versus the nigrostriatal system (Di Giovanni et al., 2006; Di Giovanni and De Deurwaerdere, 2016). Moreover, the analysis of the pharmacological profile of some atypical antipsychotics led to 5-HT_{2C} receptor blockade as a valuable strategy to improve the efficacy of dopamine antagonists in long-term treatments (Meltzer, 1999). Vabicaserin is a novel antipsychotic and anorectic agent with high agonist efficacy at the 5-HT_{2C} receptor (Dunlop et al., 2011) and has been shown to be effective in treating schizophrenia, improving positive symptoms (Shen et al., 2014). Unfortunately, the clinical development of vabicaserin by Pfizer was terminated because the drug failed to meet the primary efficacy end point in clinical trials (https://clinicaltrials.gov/ct2/show/results/NCT00563706?term=vabicaserin&rank=2).

Sertindole, a newer antipsychotic (Juruena et al., 2011), alternatively exerts a potent inverse agonist activity at the 5-HT_{2C} receptor (Herrick-Davis et al., 2000), along with dopamine D_2, α_1-adrenergic receptor and 5-HT_{2A} receptor blockade (Hietala et al., 2001). Sertindole is effective in reducing anxiety and improving cognition/memory and brain plasticity, most probably by reducing 5-HT_{2C} receptor tonic activation (Hietala et al., 2001).

Several lines of evidence have identified the 5-HT_{2C} receptor in the origin of some side effects, both motor and metabolic, associated with the chronic use of antipsychotic drugs. For instance, 5-HT_{2C} receptor blockade seems to contribute to AIWG, one of the most common and debilitating side effects induced by chronic treatment with these medications (Reynolds et al., 2005; Shams and Muller, 2014). Therefore, 5-HT_{2C} receptor agonists may have an antipsychotic activity without inducing AIWG and alterations of glucose homeostasis caused by atypical antipsychotics. However, agomelatine as a 5-HT_{2C} receptor antagonist has a more favorable profile and does not influence body weight in depressed patients (Pompili et al., 2013).

Other important side effects induced by the chronic use of antipsychotics that may be related to the activation of the 5-HT_{2C} receptor are the movement disorders, such as dystonia, acute Parkinsonism, and tardive dyskinesia, referred to globally as antipsychotic-induced extrapyramidal side effects (EPS) (Tarsy and Baldessarini, 1984; Tarsy et al., 2002; Janno et al., 2004). The inverse agonism at the 5-HT_{2C} receptor evoked by some atypical antipsychotics might indeed explain their fewer EPS effects (Herrick-Davis et al., 2000). Consistently, ritanserin, a nonselective 5-HT_2 antagonist, limits the occurrence of EPS in patients treated with typical neuroleptic drugs (Bersani et al., 1990). Moreover, the affinity of typical antipsychotics toward the 5-HT_{2C} receptor inversely correlates to the EPS severity (Richtand et al., 2007; Richtand et al., 2008). Nevertheless, experimental evidence suggests that concurrent 5-HT_{2C} receptor agonism might

increase the efficacy of typical and atypical antipsychotics, allowing dose-sparing with a reduction of side effects (Grauer et al., 2009).

4. Mood Disorders and Anxiety. Depression and anxiety are complex illnesses that have in common an altered central 5-HT tone. Compelling evidence accumulating over more than four decades has indicated the 5-HT_{2C} receptor is critically involved in the serotonergic regulation of these pathologic states. Indeed, mCPP and MK-212 induce anxiogenic-like behaviors in animals (Kennett et al., 1989; Benjamin et al., 1990; Shepherd et al., 1994; Bilkei-Gorzo et al., 1998; Millan, 2005; Martin et al., 2013) and induce anxiety and panic when administered in humans (Lowy and Meltzer, 1988; Kahn and Wetzler, 1991; Sevy et al., 1994; Southwick et al., 1997; Gatch, 2003). These anxiogenic effects are likely due to the activation of the 5-HT_{2C} receptor. In agreement, Tecott and colleagues showed that 5-HT_{2C} receptor knockout mice exhibit an anxiolytic-like phenotype not attributable to locomotor alterations (Heisler et al., 2007b). Moreover, desensitization of the 5-HT_{2C} receptor in SERT knockout mice has been shown to contribute to the moderation of the anxiety phenotype (Martin et al., 2014b5) and to the antidepressant effects (Prisco and Esposito, 1995; Di Giovanni et al., 2006). On the other hand, mCPP induced anxiolytic effects in mice (Nic Dhonnchadha et al., 2003) but showed antidepressant-like properties in the anhedonia model in rats (Moreau et al., 1996) and was recently observed to be anorexigenic without inducing anxiety/depression in humans (Thomas et al., 2014). Moreover, RO60-0175 presented an antidepressant profile or an anxiolytic/anticompulsive profile in some tests (Cryan and Lucki, 2000; Nic Dhonnchadha et al., 2003). Interestingly, anxiogenic responses induced by RO60-0175 (Martin et al., 2013, 2014a) are sometimes related to its sedative properties (Kennett et al., 2000). CP809101 is ineffective in some models (Siuciak et al., 2007) and anxiogenic in others (Strong et al., 2009, 2011; Christianson et al., 2010).

Consistent with proposed anxiogenic effects of 5-HT_{2C} receptor activation, 5-HT_{2C} receptor antagonists display anxiolytic/antidepressant properties in numerous tests (Kennett et al., 1994, 1996, 1997; Wood et al., 2001; Millan, 2005; Harada et al., 2006). Interestingly, there are models in which both 5-HT_{2C} receptor agonists and antagonists display anxiolytic/antidepressant properties, including the chronic mild stress–induced anhedonia model and the activity consequent to olfactory bulbectomy. The tendency is that selective 5-HT_{2C} receptor agonists would be more appropriate in the treatment of depression, obsessive-compulsive disorder (OCD), or panic attacks, whereas the antagonists would be better suited for generalized anxiety and obsessive-compulsive disorder (Jenck et al., 1998; Millan, 2003, 2005). On the contrary, the atypical antidepressants mirtazapine and mianserin (Hayasaka et al., 2015) and a recently developed new antidepressant agomelatine (Millan et al., 2003, 2011; Millan, 2005) have clear antagonistic 5-HT_{2C} receptor profiles. The lack of selectivity of the pharmacological 5-HT_{2C} receptor tools employed to date as well as the limited appreciation of 5-HT_{2C} receptor function and its unique molecular mechanisms (e.g., edited and spliced 5-HT_{2C} receptor isoforms, constitutive activity, and biased signaling) complicate final conclusions at present.

The paradoxical efficacy of both 5-HT_{2C} receptor agonists and antagonists is likely due to the complex neurobiological basis of depression/anxiety and the fact that different behavioral responses involve different areas expressing the 5-HT_{2C} receptor, of which activation produces opposite effects (Millan et al., 2005). Indeed, local activation of the 5-HT_{2C} receptor in the basolateral part of the amygdala induces anxiety (Campbell and Merchant, 2003), whereas the activation of those in the dorsal periaqueducal gray triggers anxiolytic responses (Yamashita et al., 2011). Thus, the long-term antidepressant effect of compounds such as SSRIs may be related to a region-dependent desensitization of the 5-HT_{2C} receptor in determined regions (Prisco and Esposito, 1995; Di Giovanni et al., 2006; Martin et al., 2014a).

In summary, selective 5-HT_{2C} receptor antagonists may present promising molecules for developing new antidepressant/anxiolytic drugs. These could be considered as monotherapy or for augmentation strategies to improve other antidepressant responses (Cremers et al., 2004, 2007) with the ability to reverse several SSRI-induced side effects.

5. Epilepsy. Early evidence implicated a 5-HT involvement in the pathophysiology of epilepsy (Bonnycastle et al., 1957). Since then, evidence has accumulated clearly showing that there is a direct link between 5-HT levels and epilepsy. Increasing 5-HT levels in the CNS is generally antiepileptic, whereas a decrease favors epileptogenesis and seizure generation [for reviews, see Bagdy et al. (2007) and Guiard and Di Giovanni (2015)]. Moreover, a common 5-HT dysfunction might underlie both epilepsy and comorbid depression seen in patients with epilepsy (Kanner et al., 2012; Guiard and Di Giovanni, 2015).

The 5-HT_{2C} receptor is thought to be involved in seizure generation and cell excitability (Jakus et al., 2003; Isaac, 2005). Tecott and colleagues showed that 5-HT_{2C} receptor knockout mice display spontaneous convulsive generalized seizures, which cause their high mortality rate (Tecott et al., 1995) and a reduced threshold for various convulsing stimuli (Applegate and Tecott, 1998; Heisler et al., 1998b). Conversely, 5-HT_{2C} receptor activation increased the threshold of general convulsion induced by pentylenetetrazole and electroshock in mice (Upton et al., 1998). The 5-HT_{2C} receptor also negatively controls nonconvulsive generalized seizures. Di Giovanni and colleagues showed that, in the polygenic animal model of absence epilepsy, the Genetic Absence Epilepsy

Rat from Strasbourg (Danober et al., 1998), RO60-0175 (unpublished data), lorcaserin, and CP809101 were capable of blocking spike and wave discharges (Venzi et al., 2016). Interestingly, as expected, SB242084 blocked the effect of lorcaserin and CP809101 but also showed some antiabsence effects. One possible mechanism by which 5-HT_{2C} receptor activation exerts antiepileptic effects is via the normalization of the aberrant $GABA_A$ receptor tonic inhibition in the ventrobasal thalamus seen in different animal models of absence epilepsy (Cope et al., 2009; Crunelli and Di Giovanni, 2014, 2015). Findings with the Genetic Absence Epilepsy Rat from Strasbourg animal model of absence epilepsy are in agreement with those obtained in another model of absence epilepsy, the Wistar Albino Glaxo/Rij-rat, in which mCPP decreases the cumulative duration of spike and wave discharges via the activation of the 5-HT_{2C} receptor (Jakus et al., 2003; Jakus and Bagdy, 2011).

The 5-HT_{2C} receptor system seems devoid of any modulatory role in partial seizures or, paradoxically, has a proepileptic role in this type of epilepsy. Indeed, observations from Di Giovanni's group show that mCPP and lorcaserin, but not RO60-0175, were able to halt hippocampal after discharges in a rat model of temporal lobe epilepsy, an effect potentiated and insensitive to SB242084 pretreatment (Orban et al., 2014). These data indicate that other 5-HT receptors are involved in the antiepileptic effect of mCPP and lorcaserin, probably the 5-$HT_{1A/7}$ receptor (Orban et al., 2013) or unknown targets, confirming previous findings (Damjanoska et al., 2003; Navailles et al., 2013a; Orban et al., 2014).

In summary, 5-HT_{2C} receptor agonists may have new therapeutic utility in epilepsy. In particular, the FDA-approved lorcaserin may be useful for the treatment of human generalized convulsive and nonconvulsive epilepsy, which is very important in consideration of the fact that the epileptic drug pipeline is limited. Moreover, activation of the 5-HT_{2C} receptor may also be useful for treating comorbid neuropsychiatric comorbidity commonly seen in patients with epilepsy (Di Giovanni and De Deurwaerdere, 2016; Venzi et al., 2016).

6. Sleep. A role for 5-HT in the sleep-wake cycle is well documented, as 5-HT has been shown to promote wakefulness and reduce REM sleep [for review, see Monti (2011)]. In general, nonselective 5-$HT_{2A/2C}$ receptor agonists and selective 5-HT_{2C} receptor agonists increase wakefulness and decrease SW and/or REM sleep following systemic administration in rats, whereas nonselective 5-$HT_{2A/2C}$ receptor antagonists and selective 5-HT_{2C} receptor antagonists tend to decrease wakefulness and promote SW with reduced REM sleep [for review, see Monti (2011)]. In contrast to what may be anticipated, 5-HT_{2C} receptor knockout mice display increased wakefulness with reduced SW and non-REM sleep (Frank et al., 2002). The increased wakefulness and reduced SW sleep have been attributed to compensatory mechanisms, possibly including the enhanced dopaminergic and adrenergic neurotransmission that occurs as a result of the constitutive knockout of the 5-HT_{2C} receptor (Frank et al., 2002).

The nonselective 5-$HT_{2C/2A}$ receptor antagonists ritanserin and ketanserin enhanced SW sleep in a dose-dependent manner in human subjects with normal sleep patterns (Sharpley et al., 1990; Idzikowski et al., 1991). Similar results were observed following treatment with seganserin, ICI169369, and SR46349B (Dijk et al., 1989; Landolt et al., 1999). In addition, ritanserin has been reported to improve SW sleep in subjects with insomnia (Adam and Oswald, 1989), major depression (Staner et al., 1992), and generalized anxiety disorder (da Roza Davis et al., 1992). In patients with schizophrenia, typical and atypical antipsychotic drugs with nonselective 5-$HT_{2C/2A}$ receptor antagonist properties tend to increase total sleep time and efficiency but reduce the latency and duration of REM sleep (Taylor et al., 1991; Nofzinger et al., 1993; Wetter et al., 1996; Sharpley et al., 2000; Muller et al., 2004). Taken together, these results indicate an involvement of the 5-HT_{2C} receptor in the maintenance of normal sleep architecture and suggest possible avenues for future research and medication development for the treatment of insomnia.

7. Clinical Impact of RNA Editing of the 5-HT_{2C} Receptor. Many studies have attempted to analyze 5-HT_{2C} receptor RNA editing profiles in postmortem samples from patients with psychiatric disorders. The majority of these reported that 5-HT_{2C} receptor RNA editing in postmortem prefrontal cortex samples from patients with schizophrenia or bipolar disorder is not altered (Niswender et al., 2001; Dracheva et al., 2003, 2008b; Iwamoto and Kato, 2003; Zhu et al., 2012). In contrast, mixed results were obtained from postmortem prefrontal cortex samples from patients with major depression, with some studies reporting no change (Niswender et al., 2001; Zhu et al., 2012; Lyddon et al., 2013) and other studies reporting a decrease (Gurevich et al., 2002b) or increase in 5-HT_{2C} receptor RNA editing (Iwamoto and Kato, 2003). Several studies have reported that psychiatric patients who committed suicide had increased 5-HT_{2C} receptor RNA editing with increased levels of the less-active 5-HT_{2C} receptor isoforms (Niswender et al., 2001; Gurevich et al., 2002b; Iwamoto and Kato, 2003; Dracheva et al., 2008b; Lyddon et al., 2013; Di Narzo et al., 2014) as well as increased expression of ADAR1 (Simmons et al., 2010). However, there were notable differences in the patterns of 5-HT_{2C} receptor RNA editing that were reported in these studies. In a similar fashion, there is great variation in the reported effects of antipsychotic and antidepressant medications on RNA editing (Sodhi et al., 2005; Abbas et al., 2010; Iwamoto et al., 2011; Martin et al., 2014a) and the effect of altered synaptic 5-HT levels on 5-HT_{2C} receptor RNA editing (Gurevich

et al., 2002a; Abbas et al., 2010; Lyddon et al., 2010; Moya et al., 2011). These variations in results highlight the requirement of a large sample size and the need to analyze hundreds of sequences per sample to obtain an accurate profile of all possible RNA editing events. In some of the more recent studies, the statistical power of the results has been increased by employing high-throughput sequencing methods that allow larger numbers of samples to be processed, in a more timely and less expensive manner, along with the ability to analyze hundreds or even thousands of sequences from a single sample (Lanfranco et al., 2009; Abbas et al., 2010; Morabito et al., 2010b; O'Neil and Emeson, 2012; Zhu et al., 2012; Lyddon et al., 2013; Anastasio et al., 2014b; Di Narzo et al., 2014). Although these studies have reported changes in 5-HT_{2C} receptor RNA editing patterns following chronic drug treatment in rodent models or changes in editing in patients with psychiatric disorders who committed suicide, the magnitude of the observed change is small, typically less than 10%. This raises several important questions. Could such a small change in 5-HT_{2C} receptor editing be physiologically relevant? Could there be larger changes in 5-HT_{2C} receptor RNA editing occurring within discrete neuronal populations, and could these changes be underestimated or masked when sampling an entire brain region?

A few of the studies described in the preceding paragraph examined RNA editing in both cortical and subcortical regions, whereas many studies examined RNA editing only in the prefrontal cortex of patients with psychiatric illnesses. Because the 5-HT_{2C} receptor is located within mesolimbic and mesostriatal regions that have been shown to regulate the activity of ascending dopamine, it still remains possible that changes in RNA editing occurring within discrete populations of neurons in cortical and/or subcortical regions could play a role in the etiology of psychiatric disorders. Future studies capable of examining RNA editing profiles of individual neuronal populations within localized subregions will provide a more accurate picture of the relationship between 5-HT_{2C} receptor RNA editing, 5-HT_{2C} receptor isoform expression, and the functional regulation of 5-HT and dopamine neurotransmission in psychiatric disorders.

X. 5-HT_3 Receptor

A. Introduction

The 5-HT_3 receptor is a Cys-loop ligand-gated ion channel, which is structurally and functionally distinct from the other six classes of 5-HT receptors whose metabotropic actions are mediated via G proteins. 5-HT_3 receptors are pentameric assemblies of five identical or nonidentical subunits that pseudosymmetrically surround the ion pore (Boess et al., 1992; Green et al., 1995). Each subunit has a large extracellular domain (ECD), four transmembrane domains (TMD I–IV), and an intracellular domain (ICD) between M3 and M4.

The ECD contains the agonist binding site, which is located at the interface of two adjacent subunits and is formed by three loops (A–C) from one (the principal) subunit and three β-strands (referred to as loops D–F) from the adjacent or complementary subunit; key residues that contribute to the binding pocket in these loops have been identified from structural data (Hassaine et al., 2014), and these are supported by a range of functional studies [for reviews, see Barnes et al. (2009) and Thompson et al. (2010)]. The binding site contains the aromatic box, which is found in all Cys-loop receptors, and in the homomeric 5-HT_3A receptor, is constituted of W183 (loop B), W90 (loop D), Y153 (loop E), F226 (loop C), and Y234 (loop C).

The transmembrane domain of each 5-HT_3 receptor subunit is composed of four (M1–M4) transmembrane α-helices, with short loops between M1 and M2 (intracellular) and M2 and M3 (extracellular). The M2 α-helices line the ion pore, and pore-facing residues contribute to ion flux and selectivity. M1, M3, and M4 all protect M2 from the surrounding membrane lipids and play a role in receptor function. The conserved proline in M1, for example, is essential for activation, and the receptor is expressed but cannot function when this proline is replaced by alanine, glycine, or leucine (Dang et al., 2000). However, substitution with non-canonical amino acids that lack hydrogen bond donor activity yields active channels similar to wild-type receptors. These suggest flexibility in secondary structure in this region of M1 is a key element in channel gating.

The ICD is formed primarily by the large M3–M4 intracellular loop; this region is responsible for receptor modulation and also plays a role in trafficking. Deletion studies reveal the ICD is not essential, as the mouse 5-HT3A receptor subunit ICD can be replaced by the heptapeptide M3–M4 linker of GLIC without loss of function (Jansen et al., 2008). Further evidence that the ICD can function as a separate domain comes from studies in which it was added to the GLIC linker peptide, resulting in modification of function by the intracellular protein RIC-3 (Goyal et al., 2011).

ICD structural details are sparse, but each subunit is known to possess an α-helix, which contributes to openings, known as portals, just below the level of the membrane. The residues that line these portals are important for ion conductance; when the 5-HT3A subunit residues are replaced with those found in the 5-HT3B subunit, the single channel conductance, which is very low in the homomeric 5-HT_3A receptor, is increased to that of the heteromeric 5-HT_3AB receptor (Kelley et al., 2003b).

Only 5-HT3A subunits can form functional homomeric 5-HT_3 receptors. These subunits have been cloned from a range of species, including human hippocampus,

amygdala, and colon (Belelli et al., 1995; Miyake et al., 1995); guinea pig small intestine (Lankiewicz et al., 1998); ferret colon (Mochizuki et al., 2000); and dog brain (Jensen et al., 2006); however, homologs are absent from invertebrates (although there is a related 5-HT–gated anion selective receptor). Multiple isoform of 5-HT3A subunits are known. Alternative splicing of the transcript encoding guinea pig, mouse, and rat, (but not dog, ferret, or human) 5-HT3A subunits results in "long" [5-HT3A(a)] and short [5-HT3A(b)] isoforms, where the 5-HT3A(b) isoform lacks five or six amino acid residues within the M3–M4 intracellular loop, which results in some subtle differences in receptor properties (Hope et al., 1993; Lankiewicz et al., 1998). In the human 5-HT3A subunit, three different splice variants have been described. Two of these (5-HT3AL and HT3Rext) would result in larger proteins, whereas the other (5-HT3AT) codes for a truncated subunit containing only a single transmembrane domain. 5-HT3Rext has not yet been functionally evaluated, but 5-HT3AT and 5-HT3AL, while not functional when expressed alone, form receptors with modified functional properties when coexpressed with canonical 5-HT3A subunits (Brüss et al., 2000).

A range of other 5-HT_3 receptor subunits have been identified (B–E), of which only the 5-HT3B subunit has been extensively investigated (Davies et al., 1999; Dubin et al., 1999; Niesler et al., 2003). Coexpression of this subunit with the 5-HT3A subunit to yield hetermeric 5-HT_3AB receptors resulted in functional receptors with properties that more closely represented those found in some native neuronal receptors, including relatively high single-channel conductance and relatively low Ca^{2+} permeability compared with homomeric 5-HT_3A receptors.

The stoichiometry of heteromeric receptors is still not clear, although the presence of at least one 5-HT_3A subunit appears to be obligatory in heteromeric receptors (Niesler et al., 2008; Holbrook et al., 2009). 5-HT_3AB receptors were originally suggested to possess a 3B:2A subunit ratio, and using atomic force microscopy with tagged subunits indicated a BABBA arrangement (Barrera et al., 2005). The physiologic relevance of this, however, has been questioned in more recent studies, in which the presence of an AA interface was clearly demonstrated (Lochner and Lummis, 2010; Thompson et al., 2011b), and fluorescently tagged subunits indicate a 3A:2B arrangement at the plasma membrane (Miles et al., 2013). This is also consistent with the near identical orthosteric binding site pharmacology when comparing h5-HT_3A and h5-HT_3AB receptors (Brady et al., 2001). The arrangement and number of 5-HT3C, 5-HT3D, and 5-HT3E subunits in functional receptors has not yet been determined, although there are many potential 5-HT_3 receptor isoforms arising from the utilization of multiple subunits in different combinations.

For more information of the structure of the 5-HT_3 receptor, see *XVI. B. 5-HT Ligand-Gated Ion Channels*.

B. Expression

5-HT_3 receptors are located in many brain areas, including the hippocampus (Fig. 15), entorhinal cortex, frontal cortex, cingulated cortex, dorsal horn ganglia, amygdala, nucleus accumbens, substantia nigra, and ventral tegmental area (Parker et al., 1996a; Barnes et al., 2009). The dorsal vagal complex in the brainstem, which is key to the vomiting reflex and contains the area postrema and nucleus tractus solitarius, has the highest levels, consistent with the potent antiemetic properties of 5-HT_3 receptor antagonists (Pratt et al., 1990; Parker et al., 1996; Fig. 16).

The functional studies are supported by expression studies, with 5-HT_3A receptor mRNA and protein being observed in regions of the CNS known to have 5-HT_3 receptors. Such studies also indicate 5-HT_3 receptor expression in a wide range of other tissues, including peripheral and sensory ganglia and the gastrointestinal tract (e.g., Michel et al., 2005; Barnes et al., 2009; Fig. 17). In addition, expression of the 5-HT3A subunit has been reported in immune cells such as monocytes, chondrocytes, T cells, synovial tissue, and platelets (Fiebich et al., 2004; Stratz et al., 2008).

There was initial controversy as to the presence of 5-HT3B subunits in brain, but later studies show it is expressed here (e.g., Brady et al., 2007), with a preference for distinct "brain-type" isoforms. The longer canonical 5-HT3B subunit is broadly expressed in many tissues, including kidney, liver, and the gastrointestinal tract, with relatively high levels in spleen, colon, small intestine, and kidney (Tzvetkov et al., 2007; Holbrook et al., 2009).

5-HT3C, 5-HT3D, and 5-HT3E receptor subunits were first identified in humans, and genes for these proteins have now been shown to exist in a range of species, although not in rodents (Niesler et al., 2008; Holbrook et al., 2009). Initial studies suggested that the 5-HT3D and 5-HT3E subunits had a very restricted expression in the GI tract, but more recent data suggests all these subunits have a relatively widespread distribution. Studies examining protein levels have lagged behind the genetic work, but expression of 5-HT3C, 5-HT3D, and 5-HT3E subunits at the protein level in the GI tract has recently been confirmed (Kapeller et al., 2011).

C. Post-translational Modifications

The human 5-HT3A subunit has four consensus sequence *N*-glycosylation sites in the N-terminal ECD domain and all can be *N*-glycosylated (Monk et al., 2004). *N*-glycosylation is essential for export from the ER, cell surface expression, and radioligand binding, although it is not necessary to preserve a ligand binding site once the receptor has matured (Green

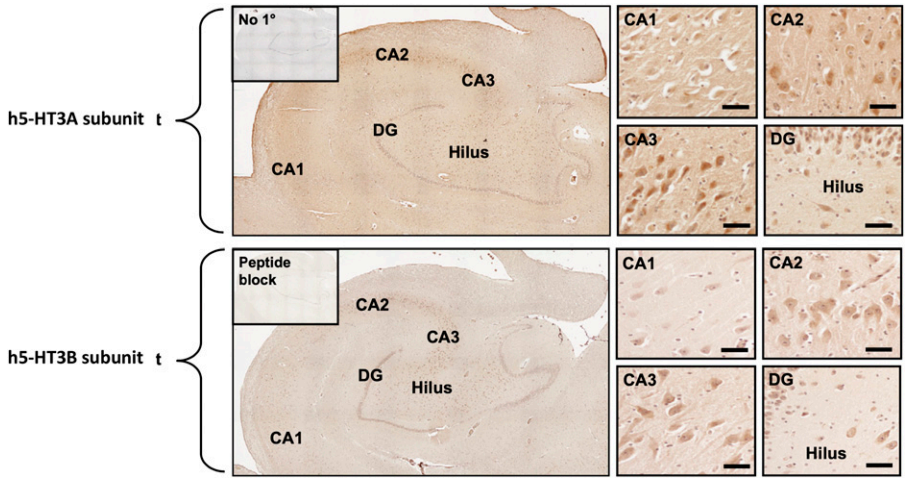

Fig. 15. Immunohistochemical detection of 5-HT3A and 5-HT3B receptor subunit expression in human hippocampus. 5-HT3A and 5-HT3B subunit immunoreactivity brown immunoreaction product in whole hippocampal section (large plates) is shown with fields of the hippocampus CA1-CA3, hilus (CA4), and dentate gyrus (DG), which are also shown at higher magnification (small plates; Scale bar, 50 μm). Immunoreactivity was not detected when either primary antibody was replaced by preimmune serum (No 1°) or following preabsorption with the immunizing peptide (peptide block). Subsequent to the detection of immunoreactivity, sections were histologically stained with hematoxylin to aid identification of hippocampal fields. Adapted from Brady et al. (2007) (with permission).

et al., 1995; Boyd et al., 2003; Quirk et al., 2004). Three of the four *N*-glycosylation sites are conserved between a range of species (N104, N170, and N186) and appear to be critical, whereas the *N*-glycosylation site at residue 28 is less important and indeed absent in rodents (Monk et al., 2004).

The other subunits have been less well studied, but it has been shown that 5-HT3B receptor subunits, when expressed alone, fail to exit the ER, providing a possible explanation as to why these subunits cannot form functional homomeric receptors. ER retention is due, at least in part, to a CRAR retention motif, which forms part of the M1–M2 intracellular loop (Boyd et al., 2003). Coexpression with the 5-HT3A subunit may shield this ER retention motif, allowing heteromeric 5-HT_3AB receptors to reach the cell surface. There is some evidence that the 5-HT3B subunit forces a preference for expression of the heteromeric 5-HT_3 receptor, as coexpression of the 5-HT3A and 5-HT3B subunits in tsA-201 cells did not indicate the presence of homomeric 5-HT3A receptors (Barrera et al., 2005), although the physiologic relevance of this finding is not yet clear.

BiP, calnexin, and RIC-3 have been identified as ER chaperone proteins that associate with the 5-HT_3 receptor and are likely to promote correct folding, oligomerisation, post-translational modification, and/or export from the ER (Boyd et al., 2003). RIC-3 has been the most widely studied but has different effects depending on the subunits, the species they originated from, and the expression system (Castillo et al., 2005; Cheng et al., 2005, 2007). Expression of human homomeric 5-HT_3A receptors in transfected mammalian cells, for example, is enhanced by RIC-3, but it causes inhibition of heteromeric (5-HT_3AB) receptor expression, and mouse 5-HT_3A receptor expression in oocytes is completely abolished (Cheng et al., 2005). This apparent discrepancy may be due to other proteins that could influence 5-HT_3 receptor expression. Cyclophillin A, for example, promotes 5-HT_3A receptor expression in the cell membrane via an integral peptidyl prolyl isomerase activity (Helekar and Patrick, 1997), and there may be a range of other proteins yet to be identified that can modify 5-HT_3 receptor expression.

Similar to the human 5-HT3A subunit, the human 5-HT3B subunit has a number of consensus sequences for *N*-glycosylation sites in the N-terminal ECD domain (five; Massoura et al., 2011), and all can be *N*-glycosylated (Massoura et al., 2011). *N*-glycosylation of the human 5-HT3B subunit at each of the five consensus sites facilitates export from the ER and efficient cell surface expression when the 5-HT3B subunit is coexpressed with the human 5-HT3A subunit to generate hetermeric 5-HT_3AB receptors (Massoura et al., 2011).

D. Pharmacology

There are many selective and potent compounds that act at 5-HT_3 receptors. Many 5-HT_3 receptor agonists have in common a basic amine, an aromatic ring, a hydrophobic group, and two hydrogen bond acceptors; potent agonists include 2-methyl-5-HT, phenylbiguanide, and m-chlorophenylbiguanide (Kilpatrick et al., 1990; Cockcroft et al., 1995). Data from AChBP suggest that agonists tend to be relatively small compounds, as they need to allow the C loop to close over the binding site, initiating the gating process; the structure of 5-HT binding protein (5-HTBP),

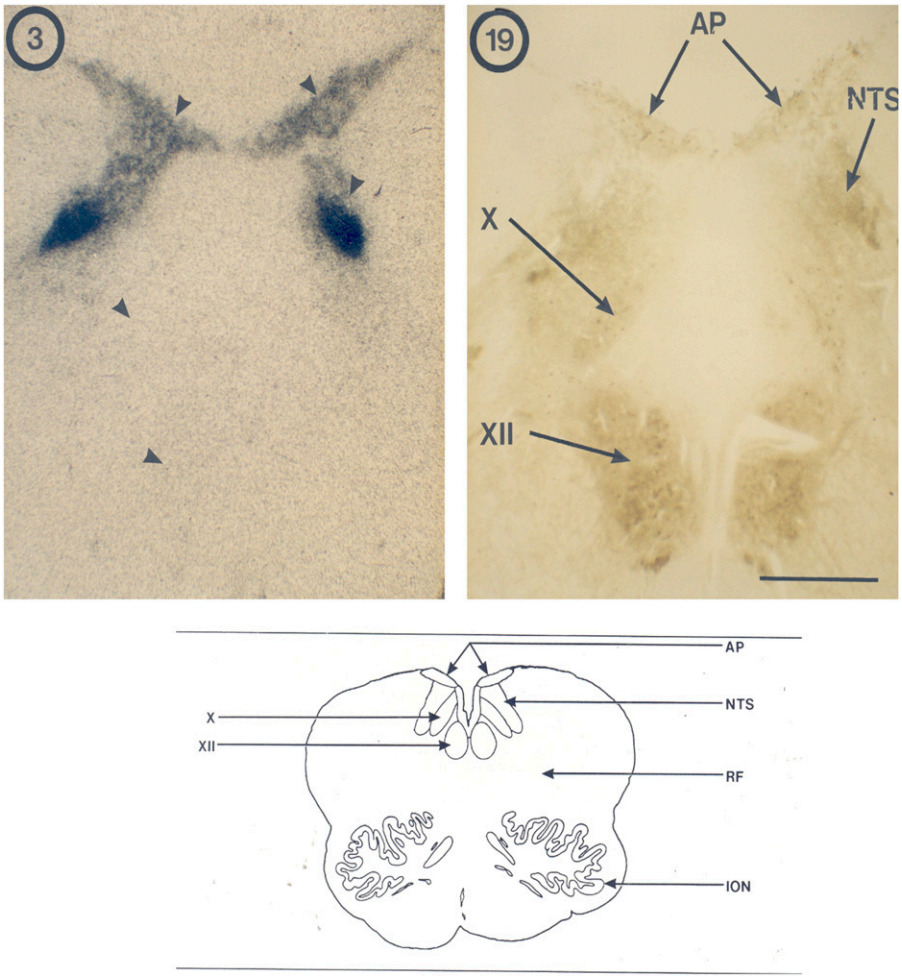

Fig. 16. 5-HT_3 receptor autoradiography in the human brainstem. Left: [^{3}H]-(S)-zacopride binding to the 5-HT_3 receptor in coronal section of human brainstem at the level of the chemoreceptor trigger zone (AP, area postrema; NTS, nucleus tractus solitarius; X, dorsal motor nucleus of the vagus nerve). Right: Adjacent section stained for acetylcholinesterase (hypoglossal nerve nucleus). [^{3}H]-(S)-zacopride binding in the presence of ondansetron (1.0 μM) was absent (data not shown).

a modified version of AChBP, which binds 5-HT and granisetron, supports a similar mechanism of action for the 5-HT_3 receptor (Tsetlin and Hucho, 2009; Kesters et al., 2013).

A range of highly selective 5-HT_3 receptor partial agonists with a range of intrinsic activities are available (e.g., Manning et al., 2011, 2014; Roberts et al., 2020) that have been proposed as therapeutics to treat the symptoms of, for example, irritable bowel syndrome (IBS) with diarrhea (IBS-D) and carcinoid syndrome (Roberts et al., 2020).

5-HT_3 receptor competitive antagonists, which bind at the orthosteric (agonist) binding site, are usually larger than agonists; they require an aromatic part, a basic moiety, and an intervening hydrogen bond acceptor. For most antagonists, these are a rigid aromatic or heteroaromatic ring system, a basic amine, and a carbonyl group (or isosteric equivalent) that is coplanar to the aromatic system (Evans et al., 1991), and there are slightly longer distances between the aromatic and amine group when compared with the agonist pharmacophore. Only small substituents, such as a methyl group, can be accommodated on the charged amine (Schmidt and Peroutka, 1989). Many potent antagonists of 5-HT_3 receptors have 6.5 heterocyclic rings, and the most potent compounds contain an aromatic six-membered ring. Morphine and cocaine (Gaddum and Picarelli, 1957) were the first antagonists used to characterize the 5-HT_3 receptor, with more selective 5-HT_3 receptor antagonists being developed in the 1980s, including MDL72222 or bemestron (Fozard, 1984) and ICS 205-930 or tropisetron (Donatsch et al., 1985). Other compounds that were developed include ondansetron, granisetron, and zacopride, which act at nanomolar concentrations, and there is now a wide range of similarly potent compounds, with many containing bicyclic heteroaromatic structures, that is, quinoxalines, quinazolines, or quinolines (Verheij et al., 2012). Data indicate that these compounds, and therefore possibly all ligands that bind to the orthosteric site, are stabilized in the binding pocket by interaction with water molecules.

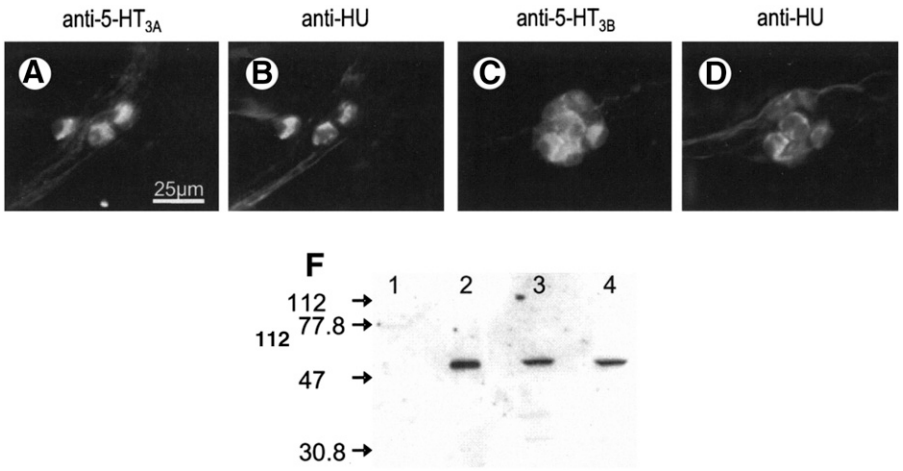

Fig. 17. 5-HT_3 receptor protein expression (5-HT3A and 5-HT3B subunits) in human gut. (A) Immunolabeling of a ganglion in the human submucous plexus with an antibody against the 5-HT3A subunit. (B) The same ganglion as in (A) stained with an antibody against the human neuronal protein HuC/HuD, which labels all neurons. All anti-HuC/HuD–positive neurons are immunoreactive for the 5-HT3A antibody. (C) Immunolabeling of another human submucous ganglion with an antibody against the 5-HT3B subunit. (D) The same ganglion as in (C) stained with anti-HuC/HuD. All anti-HuC/HuD–positive neurons were immunoreactive for the 5-HT3B antibody. Scale bar, 25 μm (A–D). (F) Detection of h5-HT3B immunoreactivity by SDS polyacrylamide gel electrophoresis/Western blot. Lane 1, HEK293 cells stably expressing with h5-HT3A subunit; lane 2, HEK293 cells stably expressing both the h5-HT3A and h5-HT3B subunits; lane 3, human large intestine; lane 4, human small intestine. Numbers are the positions of the molecular weight markers (kilodalton). Adapted from Michel et al. (2005) (with permission).

Some 5-HT_3 receptor inhibitors act by blocking the channel; picrotoxin, which was originally considered to be relatively selective for the $GABA_A$ receptor, also blocks the 5-HT_3 receptor channel; the binding of picrotoxinin (the active component of picrotoxin) has been localized to the 6' position of M2 (Das and Dillon, 2003; Thompson et al., 2011a). Compounds structurally similar to picrotoxin, such as the gingkolides and bilobalide, act similarly (Thompson et al., 2011a). Diltiazem, which blocks voltage-gated calcium channels, is also known to block the 5-HT_3 receptor pore and acts close to the 7' and/or 12' residues in homomeric receptors (Gunthorpe and Lummis, 1999; Thompson et al., 2011a). Morphine and its analog methadone as well as the antimalarial compounds quinine and mefloquine may also exert their inhibitory effects via binding to the pore, highlighting the common mechanisms that many of these drugs share and also the promiscuity that many of these compounds display (Brady et al., 2001; Deeb et al., 2009; Baptista-Hon et al., 2012)

There are a number of allosteric modulators that effect 5-HT_3 receptor function, including n-alcohols, anesthetics, antidepressants, cannabinoids, opioids, steroids, and natural compounds; these can inhibit or enhance receptor activity, and many also modulate other Cys-loop receptors, although not always in the same direction [see reviews by Parker et al. (1996), Walstab et al. (2010), Davies (2011), and Machu (2011)]. Specific binding sites for these compounds have mostly not yet been confirmed, although some may bind in an intersubunit binding cavity at the top of the TMD (Trattnig et al., 2012). The effects of these compounds have mostly been studied on 5-HT_3A receptors [see Downie et al. (1995) and Parker et al. (1996), but see Bentley and Barnes (1998) for impact on native 5-HT_3 receptors], although alcohols and inhaled anesthetics have been shown to have reduced sensitivity at 5-HT_3AB receptors, whereas the effects of etomidate, propofol, and pentobarbital are similar at 5-HT_3A and 5-HT_3AB receptors (Solt et al., 2005; Stevens et al., 2005; Rusch et al., 2007).

A further pharmacological phenomenon termed cryptic orthosteric modulation was identified using the human 5-HT_3A receptor as a model (Powell et al., 2016); this pharmacological mechanism may convey therapeutic benefits when targeting ligand-gated ion channels (Powell et al., 2016).

E. Function

The 5-HT_3 receptor pore is a relatively nonselective cation channel, constructed of five pseudosymmetrically arranged M2 α-helices (one from each of the five subunits). The residues that line the ion-accessible face of M2 are predominantly nonpolar and are the major controlling influence on ion flux (Reeves et al., 2001; Panicker et al., 2002; McKinnon et al., 2011). Currents are primarily carried by Na^+ and K^+ ions, although divalent and small organic cations are also permeable (Derkach et al., 1989; Yang, 1990; Maricq et al., 1991).

Ionic selectively is predominantly mediated via residues in M2. A triple mutant receptor with a proline insertion at −1' and the substitutions E−1'A and V13'T resulted in an anion-permeable mouse 5-HT_3A receptor (Gunthorpe and Lummis, 2001), although subsequent studies showed that the replacement of only two residues (E–1' and S19'R) was needed to invert ion selectivity (Thompson and Lummis, 2003).

Changing −1'E alone resulted in nonselective channels, indicating that the rings of charge at either end of M2 charge make the most critical contribution (Thompson and Lummis, 2003). Equivalent residues participate in the selectivity filters of other Cys-loops (Thompson et al., 2010).

5-HT_3A receptors are almost equally permeable to monovalent and divalent cations (P_{Ca}/P_{Cs} = 1.0–1.4) (Brown et al., 1998; Davies et al., 1999; Livesey et al., 2011). However, human 5-HT_3AB receptors have lower Ca^{2+} permeability [P_{Ca}/P_{Cs} = 0.6 (Davies et al., 1999)], possibly the consequence of the 20' residue being neutral (Asn and not Asp) in human 5-HT3B subunits. Consistent with this, a D20'A substitution in 5-HT3A subunits reduces Ca^{2+}-relative permeability [P_{Ca}/P_{Cs} = 0.4 (Livesey et al., 2008)]. Recent studies suggest that the ICD may also play a role in Ca^{2+} permeability, as substitutions of charged residues in this region can have a major effect on Ca^{2+} permeability (Livesey et al., 2011).

The single-channel conductance of the homomeric receptor is low; values of 0.4–0.76 pS have been reported (Davies et al., 1999; Gunthorpe et al., 2000; Kelley et al., 2003). The presence of Lys in the M2 regions was originally considered a possible explanation, but substitutions here revealed this was not the case (Gunthorpe et al., 2000). Subsequent work revealed that the low conductance was due to Arg residues located in the amphipathic helix of the M3–M4 loop, which forms holes, known as portals, through which ions can cross between the intracellular vestibule of the receptor and the cell interior (Kelley et al., 2003).

Heteromeric 5-HT_3AB receptors show a range of distinct biophysical characteristics when compared with 5-HT3A receptors: their desensitization is more rapid, concentration-response curves reveal lower EC_{50} values and Hill slopes, their voltage dependence is linear, and their divalent cation permeability is much reduced; most noticeable, however, is the large single-channel conductance (13–16 pS) (Peters et al., 2005). This has been shown to be due to the substitution of Arg residues in the M3–M4 helical region of the 5-HT3A subunit by neutral or negative-charged residues in the 5-HT3B subunit (Kelley et al., 2003).

Other heteromeric receptors have not yet been extensively investigated, but studies to date indicate that they are more similar to homomeric 5-HT_3A receptors.

The gate of the 5-HT_3A receptor channel is located centrally within M2 (Panicker et al., 2002), consistent with the "hydrophobic girdle" model of channel gating. The hydrophobic girdle is a region in the center of M2 that is less than 3.5 Å diameter over a distance of ~8 Å; the residues that face the pore here are hydrophobic (13'Val and 9'Leu), making it effectively impermeable to ions in the closed conformation (Miyazawa et al., 2003). Data consistent with this hypothesis are substitution of Val13' residues in the 5-HT_3A receptor by threonine or serine, which caused an increase in agonist potency (Dang et al., 2000) or spontaneous channel openings (Bhattacharya et al., 2004a), and substitutions of Leu9' by a range of amino acids, which affected agonist potency and desensitization rates (Yakel et al., 1993).

F. Clinical Relevance

Studies implicate the malfunction of 5-HT_3 receptors in a range of neurologic and gastrointestinal disorders (Walstab et al., 2010; Niesler, 2011). Selective 5-HT_3 receptor antagonists (e.g., first-generation selective antagonists such as ondansetron and granisetron and second-generation antagonists such as palonosetron) have revolutionized the control of emesis, particularly in patients receiving high aggressive cytotoxic anticancer chemotherapy (e.g., cisplatin) and radiotherapy. Thus, the release of substantial amounts of 5-HT from enterochromaffin cell stores in the gastrointestinal tract as a consequence of antimitotic anticancer therapy may activate local (vagal) and central (chemoreceptor trigger zone) 5-HT_3 receptors to evoke nausea and vomiting. Simple antagonism of these 5-HT_3 receptors offers substantial relief to patients. See *XX. 5-HT Receptors and the Gastrointestinal Tract* for further discussion of the 5-HT_3 receptor and emesis.

The identification of 5-HT_3 receptors in immune cells also suggests a possible role of 5-HT_3 receptors in immunologic processes and inflammation and suggests that they may plausibly be involved in diseases such as atherosclerosis, tendomyopathies, and fibromyalgia (Fiebich et al., 2004; Stratz et al., 2008).

Some SNPs have been identified in patients with bipolar affective disorder (BPAD) or schizophrenia, disorders that segregate with cytogenetic abnormalities involving a region on chromosome 11 that harbors the HTR3A gene (Weiss et al., 1995). Further studies are needed with two SNPs found in schizophrenic patients (R344H and P391R) to determine if they contribute to disease in these patients, but a significant association was found in BPAD with a P16S mutation in the 5-HT3A subunit, with reporter constructs indicating this mutant could modulate expression levels. (Niesler et al., 2001; Thompson et al., 2006; Krzywkowski et al., 2007). Additional SNPs in the HTR3A gene result in the 5-HT3A(A33T) and 5-HT3A(M257I) subunit variants, both of which are associated with reduced levels of cell surface expression, and the 5-HT3A(S253N) variant, which does not appear to compromise plasma membrane expression (Krzywkowski et al., 2007). The significance of these is yet to be determined.

In the 5-HT3B subunit, there has been an extensive investigation into a very common SNP, Y129S, which is linked both to BPAD and major depression in women (Krzywkowski, 2006; Hammer et al., 2012). Unusually, the Y129S variant is a gain of function mutation, as 5-HT_3AB(Y129S) receptors have an increased maximal response to 5-HT, decreased desensitization and

deactivation kinetics, and a sevenfold increase in mean channel open time in comparison with heteromeric receptors containing the wild-type 5-HT3B subunit (Krzywkowski et al., 2008). An intermediate effect is apparent for receptors assembled from a mixture of wild-type 5-HT_3A, wild-type 5-HT_3B, and 5-HT_3B(Y129S) subunits, suggesting that signaling via the 5-HT_3AB receptor in heterozygous as well as homozygous individuals is altered by this SNP (Krzywkowski et al., 2008).

Studies in the more recently discovered HTR3C, HTR3D, and HTR3E genes also indicate possible involvement in disease. An SNP in the 5-HT3C gene (N163K) has been correlated with IBS, and expression studies suggest it causes an increase in receptor density (Kapeller et al., 2011). Increased expression has also been associated with an SNP in the 3′untranslated region of the HTR3E gene, also associated with IBS, which inhibits the binding of a microRNA, as described above (Kapeller et al., 2008).

IBS-D is another disorder in which 5-HT_3 receptor antagonists can be effective therapeutic agents, although side effects that may be associated with a high level of 5-HT_3 receptor inhibition supports the development of 5-HT_3 receptor partial agonists (e.g., Roberts et al., 2020). See *XX. 5-HT Receptors and the Gastrointestinal Tract* for discussion of the role of the 5-HT_3 receptor and IBS.

Additional studies suggest that a wide range of other diseases have the potential to be treated with 5-HT_3 receptor–selective drugs, including addiction, pruritis, migraine, chronic heart pain, bulimia, and neurologic phenomena such as anxiety, psychosis, nociception, and cognitive function (Thompson and Lummis, 2007; Walstab et al., 2010).

See *XVIII. 5-HT Receptors and the Brain*, *XIX. 5-HT Receptors and the Cardiovascular System*, *XX. 5-HT Receptors and the Gastrointestinal Tract*, and *XXI. 5-HT Receptors and the Immune System* for further discussion of the clinical relevance of the 5-HT_3 receptor.

XI. 5-HT_4 Receptors

A. Introduction

An atypical 5-HT receptor was initially identified to be positively coupled to adenylyl cyclase in colliculi neurons (Dumuis et al., 1988), guinea pig hippocampus (Bockaert et al., 1990), and human and porcine cardiac atria (Kaumann, 1990; Kaumann et al., 1990; Villalon et al., 1990). This receptor was insensitive to 5-HT_1 receptor and 5-HT_2 receptor antagonists and was antagonized (but with a relatively low affinity) by tropisetron (at the time thought to be a relatively selective 5-HT_3 receptor antagonist) but not by another 5-HT_3 receptor antagonist, MDL 72222. This new 5-HT receptor was named the "5-HT_4" receptor.

B. Gene and Primary Structure

The cloning of the rat cDNA by Gerald et al. (1995) revealed the first primary sequence of 5-HT_4 receptor. It is a classic GPCR with seven transmembrane domains (TMD). Two splice variant sequences were found initially [5-HT_{4S} and 5-HT_{4L}, later renamed 5-$HT_{4(a)}$ and 5-$HT_{4(b)}$] coding for proteins of 387 and 388 amino acids, respectively. The splicing occurs within the C-terminal domain following the splicing site coding for the leucine 358 (L^{358}). This splicing site at position L^{358} was subsequently found in the majority of the numerous splice variant transcripts sequenced in rodents, pig, and human (Blondel et al., 1997, 1998; Claeysen et al., 1999; Bach et al., 2001; Brattelid et al., 2004a; Bockaert et al., 2006; Coupar et al., 2007). Subsequently in human, additional splice variants differing in their C-terminal sequences following L^{358} have been identified [a, b, c (a shorter nonrelevant form of the c variant has been reported that is due to a sequencing error), d, e, f, g, i, and n; Bockaert et al., 2006; Coupar et al., 2007; Fig. 18]. For a complete analysis of intron/exon junctions of the human gene, see Bockaert et al. (2004a). Another splice variant (h) occurs in the second extracellular loop (Bender et al., 2000). In pig, at least nine other different splice variants, not described in humans, have been reported, including a functional homofusion variant (De Maeyer et al., 2008a). The coding sequence of the 5-HT_4 receptor gene (*HTR4*) extends over more than 200 kb (Genes, Ensembl release 82) and is localized on chromosome 5 (5q31-q-33) (Claeysen et al., 1997). It contains at least 14 exons. Each of the C-terminal variant sequences contains one of the exons 8–13 (Bockaert et al., 2006). The variant h is the only form that results from expression of exon 6. The presence or not of exon 1 in the 5-$HT_{4(a)}$ transcript is debated (Hodge et al., 2013), as the ATG starting site is localized in exon 2. Recently, a novel transcript has been found in the human brain in which exon 1 and 2 are substituted for a novel exon (N) containing an expected ATG starting site, the C-terminal sequence being identical to the 5-$HT_{4(b)}$ receptor splice variant (Hodge et al., 2013). The predicted protein sequence for this 5-HT_4 receptor variant should have a different N terminus sequence, as it is longer and the *N*-linked glycosylation site is at N2 instead of N7 (Hodge et al., 2013). Similar additional N-terminal exons have been identified in mouse brain and heart (Azim et al., 2012). However, further studies to clone the complete cDNA sequences and express and functionally characterize them are required before concluding on the physiologic importance of this splice variant. As some exons (such as h or i) do not present any in-frame stop codons, many additional combinations probably remain to be discovered (Brattelid et al., 2004a). SNPs within the noncoding region of *HTR4* gene have been associated with chronic obstructive pulmonary disease and asthma/airways obstruction (Repapi et al., 2010; Hodge et al., 2013). The locus presents features consistent with a regulatory region and involves SNPs that significantly alter the binding motifs of a transcription factor regulating lung development (Foxp1)

(Hodge et al., 2013). Consistent with this finding, mice devoid of the 5-HT_4 receptor have an altered baseline lung function (House et al., 2015).

C. Expression Profile

1. Central Nervous System. Brain distribution of 5-HT_4 receptors has been studied using radiolabeled antagonists [^{3}H]GR 113808, [^{125}I]SB 207710, and [^{3}H] R116712 in many species, including mouse, rat, guinea pig, monkey, and human (Eglen et al., 1995b; Bockaert et al., 1997; Bonaventure et al., 2000; Fig. 19). Generally, a heterogeneous and comparable distribution is found in the adult brain of these various species with some interspecies differences in the globus pallidus, the substantia nigra, and the interpeduncular nucleus. The highest receptor densities are found in several regions of the limbic system (islands of Calleja, olfactory bulbs, striatum, ventral pallidum, septum, hippocampus, and amygdala) or in areas belonging to several pathways such as hippocampo-habenulo-interpeduncular and striato-nigro-tectal pathways (Waeber et al., 1993, 1994, 1996; Jakeman et al., 1994). Abundant localization of 5-HT_4 receptor mRNA in the rat brain was reported in the olfactory system, striatum, medial habenula, and the hippocampus (Ullmer et al., 1996). In the rat striatum, lesion studies showed the presence of 5-HT_4 receptor on the cell bodies of neurons that project to the substantia nigra and/or globus pallidus (Compan et al., 1996), whereas in situ hybridization experiments performed in guinea pig reported a localization of 5-HT_4 receptors on the terminals of the striatopallidal and striatonigral projections (Mengod et al., 1996). In the rat brain, comparison of mRNA distribution with [^{125}I]SB 207710 binding sites confirmed that 5-HT_4 receptors are localized both somatodendritically (caudate putamen) and on axon terminals (substantia nigra and globus pallidus) (Vilaró et al., 1996). Bonaventure et al. (2000) performed a mapping

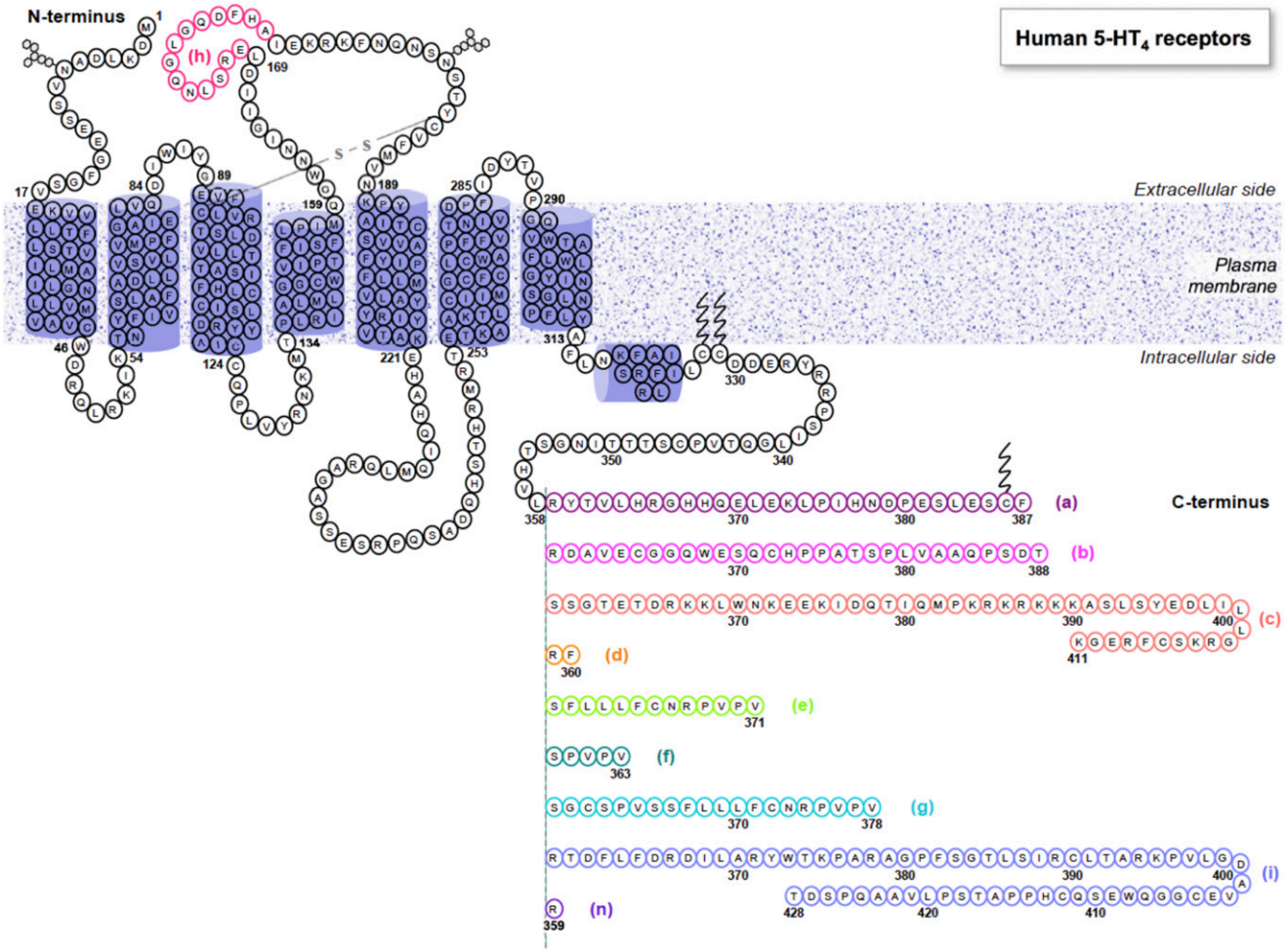

Fig. 18. Primary structure of human 5-HT_4 receptors. The amino acid sequences of the identified splice variants are depicted. 5-HT_4 receptors have identical sequences up to L^{358} and differ by the length and composition of their C-terminal domain. The 5-$HT_{4(h)}$ variant, which presents an insertion of 14 residues in the second extracellular loop, has been isolated in combination with the b isoform, and it is called 5-$HT_{4(hb)}$. N-glycosylation sites on N^7 and N^{180} are indicated. Palmytoylation sites on C^{328}, C^{329} (common to all splice variants), and C^{386} (in the a isoform) are schematized. This figure has been established in accordance with the last human genome assembly (Genes, Ensembl release 82) and modified from Padayatti et al. (2013).

analysis of both 5-HT_4 receptor mRNA and binding sites in postmortem human brains and reported combined receptor detection in caudate nucleus, putamen, nucleus accumbens, and hippocampus with moderate to low densities in the cortex. Mismatches between absence of 5-HT_4 receptor mRNA with high densities of receptor-binding sites in the globus pallidus and the substantia nigra suggested that the receptors may be localized on axonal projections originating from the striatum (Bonaventure et al., 2000).

In the cortex, hippocampus, and amygdala, 5-HT_4 receptors were described on cholinergic neurons, where they stimulate the release of acetylcholine, as well as on the glutamatergic neurons (King et al., 2008). In the striatum and nucleus accumbens, the receptors have been found on the intrinsic GABA neurons (spiny neurons) but also on glutamatergic neurons (King et al., 2008). Work using dual-label in situ hybridization found precise localization of 5-HT_4 receptors in relation with the cholinergic system. In the basal forebrain, 5-HT_4 receptor mRNA was not detected in the cholinergic cell population but in parvalbumin synthesizing and glutamatergic cells (Penas-Cazorla and Vilaro, 2015). Thus, noncholinergic cell populations within the basal forebrain, hippocampal and cortical areas that express 5-HT_4 receptors (likely glutamatergic cells) would mediate the 5-HT_4 receptor–mediated enhancement of acetylcholine (Penas-Cazorla and Vilaro, 2015) and other neurotransmitters, such as the increase in 5-HT release in the hippocampus (Ge and Barnes, 1996).

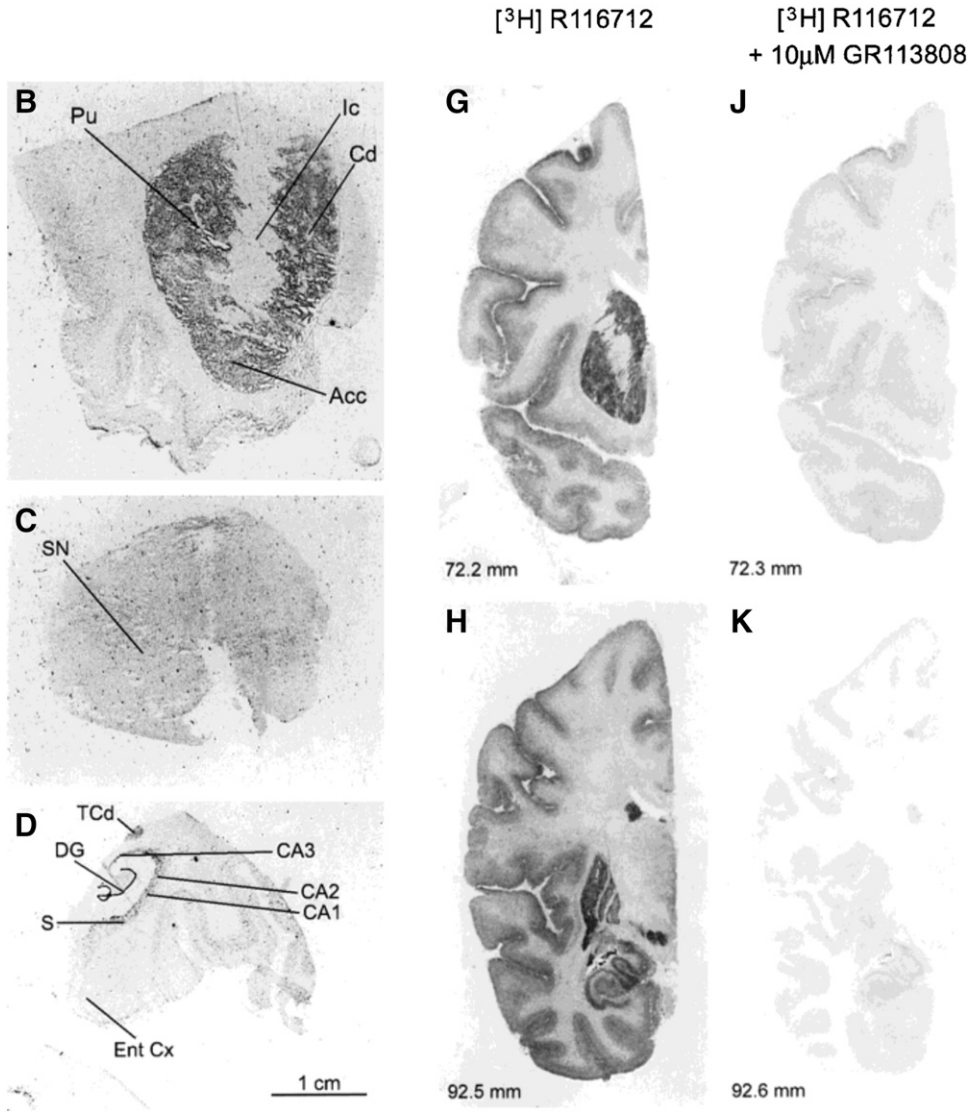

Fig. 19. 5-HT_4 receptor expression in the human brain. In situ hybridization detection of 5-HT_4 receptor mRNA (B–D) and 5-HT_4 receptor autoradiography of radioligand binding sites [[^{3}H]R116712 total binding (G and H) and nonspecific binding (J and K)]. Acc, nucleus accumbens; CA1-3, hippocampal fields; Cd, caudate nucleus; DG, dentate gyrus; Ent, Cx entorhinal cortex; Ic, internal capsule; Pu, putamen; S, subiculum; SN; no mRNA evident, substantia nigra; TCd, tail of caudate nucleus. Adapted from Bonaventure et al. (2000) (with permission).

Localization of 5-HT_4 receptor splice variants in rodents appears to be similar within brain areas and concordant to the binding studies, with a low level of 5-HT_4 receptor mRNA in the cerebellum, where no receptor-binding site is detected (Claeysen et al., 1999; Vilaro et al., 1996). A complex pattern of human 5-HT_4 receptor isoforms, which depends not only on the region studied but also on each individual brain, requires analysis of a larger cohort to be conclusive (Bender et al., 2000). However, the variations in C-terminal domain of the 5-HT_4 receptor influence its constitutive activity (Claeysen et al., 1999), internalization properties (Mnie-Filali et al., 2010), and subcellular localization via interacting partners (Joubert et al., 2004) (see 2.). The distribution of 5-$HT_{4(a)}$ receptors in the juvenile rat brain and spinal cord recently achieved using a polyclonal antibody have produced global data in accordance with the literature. However, striking staining of cerebellum cells raises a doubt on the specificity of the antibody used, which has not been tested on animals devoid of 5-HT_4 receptor (KO) (Suwa et al., 2014).

5-HT_4 receptors are also located on the respiratory Pre-Boetzinger complex in the ventrolateral medulla of the brainstem, where they modulate spontaneous respiratory activity (Manzke et al., 2003; Richter et al., 2003).

2. Periphery.

a. Heart. In porcine and human heart, 5-HT_4 receptors are mainly expressed in the atrial myocytes, and their expression was long believed to be atria-selective (Kaumann et al., 1991; Ouadid et al., 1992; Kaumann and Levy, 2006). However, expression was later also found in human and porcine ventricle (Bach et al., 2001; Brattelid et al., 2004a,b), and during heart failure and cardiac hypertrophy, their mRNA level and expression increased in the porcine, rat, and human ventricles (Brattelid et al., 2004b; Qvigstad et al., 2005a,b,c), possibly reflecting reactivation of a fetal gene expression pattern (Brattelid et al., 2012).

b. Gastrointestinal tract. In rodents, 5-HT_4 receptors are expressed in the smooth muscle of the esophagus. In many species, they are present on the intrinsic afferent neurons, motor neurons, and enterocytes of the gastrointestinal tract (Hegde and Eglen, 1996). The expression of 5-HT_4 receptors on excitatory motor neurons in the gut facilitates acetylcholine release, which stimulates gastrointestinal motility (Liu et al., 2005a; Gershon and Tack, 2007; Ren et al., 2008). In the human colon, 5-HT_4 receptors are also expressed on circular smooth muscle cells, where they induce relaxation (McLean et al., 1995). The 5-$HT_{4(h)}$ receptor variants appear to be predominantly expressed in porcine GI mucosa, suggesting their contribution to the 5-HT_4 receptor–mediated mucosal effects of 5-HT (Priem et al., 2013). In human colon, RT-PCR analysis shows that 5-$HT_{4(d)}$ and 5-$HT_{4(g)}$ receptor splice variants are significantly less likely to be detected in mucosa and longitudinal muscle compared with the 5-$HT_{4(a),\ 4(b),\ 4(c),\ 4(n)}$ splice variants (Yaakob et al., 2015).

c. Adrenal gland. 5-HT_4 receptors are present in glomerulosa and zona fasciculata of the adrenal cortex (Lefebvre et al., 1992, 1998a). An RT-PCR study demonstrated that 5-HT_4 receptors are overexpressed in adrenocortical aldosterone-producing adenoma tissues in comparison with normal adrenal cortex (Cartier et al., 2005). Moreover, a different splicing mechanism seems to occur in these tumors, where the 5-$HT_{4(a)}$ and 5-$HT_{4(b)}$ receptor splice variants, usually present in normal adrenal cortex, are absent, and the 5-$HT_{4(d)}$, a rare isoform, is over-represented (Cartier et al., 2005).

d. Salivary glands. In the rat submandibular gland, expression of 5-$HT_{4(b)}$ but not 5-$HT_{4(a)}$ receptors was demonstrated and linked to cyclic AMP formation and regulation of volume and protein content of saliva, together with 5-HT_7 receptors (Turner et al., 1996; Bourdon et al., 2000).

e. Urinary bladder. In the bladder, 5-HT_4 receptors are present on cholinergic/purinergic neurons that innervate the smooth muscle (detrusor) (Candura et al., 1996). Their stimulation with cisapride enhances human bladder contraction without affecting urethral contractions or relaxations (Kullmann et al., 2013).

f. Lung. 5-HT_4 receptor mRNA has been detected at low density in human alveolar epithelial cells type II, airway epithelial cell lines, and human airway smooth muscle (Bayer et al., 2007; Einstein et al., 2008; Hodge et al., 2013).

D. Post-translational Modifications

Post-translational modifications of 5-HT_4 receptors and their related functional consequences have been studied on receptors expressed in heterologous cells rather than native tissues.

1. N-Glycosylation. There are two putative *N*-linked glycosylation sites in the extracellular side of 5-HT_4 receptors that conform to the consensus sequence N-X-S/T, where X can be any amino acid except a Pro residue (N-terminal N7 and second extracellular loop N180; Fig. 18). The pattern of glycosylation is dependent on the nature of the cell in which 5-HT_4 receptors are expressed. Salom et al. (2012) report that the glycosylation pattern of 5-HT_4 receptors ectopically expressed in mouse rod cells in vivo is more homogeneous than the glycosylation pattern of 5-HT_4 receptors expressed artificially in HEK293 cells. The h5-$HT_{4(b)}$ receptors expressed in mouse rod cell display high-mannose–type and complex-type sugars at both N7 and N180 sites. Most of the oligosaccharides had the core structure of three mannoses (Man) and two *N*-acetylglucosamine

residues (23 species for N7 and eight species for N180). This *N*-glycosylation pattern is much more complex than for other GPCRs expressed in mouse rod cells.

2. Palmitoylation. The first crystal structure of rhodopsin reveals that after the TM7, there is a fourth intracellular loop (I-4) with α-helical structure. Thus, this I-4 loop is also called helix 8 and terminates with two palmitoyled cysteines that are well conserved in many GPCRs as in the 5-HT_4 receptors (C^{328} and C^{329}). The palmitoylation/depalmitoylation of those cysteines are associated with the wide spectrum of GPCRs biologic activities, from coupling to G proteins and regulated endocytosis to receptor phosphorylation and desensitization. Ponimaskin et al. (2002a) were the first to show that, in insect Sf9 cells, palmitoylation of the 5-$HT_{4(a)}$ is a reversible process and that agonist increases the turnover rate for receptor-bound palmitate. They identified C^{328}/C^{329} as potential acylation sites in the 5-$HT_{4(a)}$ receptor, but, in contrast to most other palmitoyled GPCRs, an additional cysteine residue C^{386}, located in the very distal portion of the C-terminal domain, was also identified as a palmitoylation site. Therefore, "complete" palmitoylation of the 5-$HT_{4(a)}$ may result in the formation of a putative additional intracellular loop (I-5). Mutation of the proximal palmitoylation sites (C^{328}S and C^{329}S) increased the capacity of the receptor to convert from the inactive (R) to the active (R*) form in the absence of agonists, whereas the mutation of C^{386}S had no effect on this parameter. 5-HT_4 receptors expressed in rod cells in vivo are palmitoylated either on C^{328} or C^{329} (Salom et al., 2012).

3. Phosphorylation. The 5-HT_4 receptor C-terminal sequence, common to all splice variants (upstream of L^{358}), contains a remarkable cluster of serine and threonine residues (S^{347}TTTINGSTHVL^{358}), which are potential phosphorylated sites for GRK. This cluster is absolutely required for β-arrestin/dynamin–dependent receptor endocytosis but not for receptor uncoupling (Barthet et al., 2005). This cluster is also required for β-arrestin binding to the receptor (Barthet et al., 2005). Like many GPCRs, 5-HT_4 receptors stimulate ERK. However, this stimulation is G_s/G_q/G_i/G_o/cAMP/PKA-independent as well as β-arrestin–independent. In contrast, this stimulation requires activation of Src tyrosine kinase (Barthet et al., 2007). This G_s-independent Src-mediated ERK activation is inhibited by GRK5, which is physically associated with the proximal region of the C terminus (upstream of the S/T cluster) (Barthet et al., 2009). Tandem mass spectra of the stimulated 5-HT_4 receptors in HEK293 cells expressing GRK5 revealed a peptide (R^{336}-R^{359}) found under a nonphosphorylated and three phosphorylated forms with one, two, and three phosphates attached, respectively (Barthet et al., 2009). Loss of phosphate on fragmentation indicated phosphorylation of S354 in the monophosphorylated peptide and the presence of an additional phosphorylated residue within the S^{347}TTT^{350} motif. The triphosphorylated peptide incorporated an additional phosphate within the S^{347}TTT^{350} motif. This indicates that GRK5 sequentially phosphorylates the peptide, first on S^{354} and then in the S^{347}TTT^{350} motif. The same 5-HT_4 receptor peptide was found to be phosphorylated in receptors expressed in mouse rods (0–5 phosphorylated residues) (Salom et al., 2012). In the same model, another peptide was found to be phosphorylated within the I-3 loop (A^{232} to R^{250}). S^{235}, S^{236}, S^{238}, and S^{242} residues were the targets for phosphorylation as well as S^{247} or T^{248} (Salom et al., 2012).

E. Pharmacology

1. Agonists. Very early, benzamides substituted by 2-methoxy-4-amino-5-chloro such as metoclopramide, renzapride, cisapride, or zacopride were identified as potent agonists of the 5-HT_4 receptors [for reviews, see Bockaert et al. (1997, 2004a, 2008) and Langlois and Fischmeister (2003)]. This was an important step in the pharmacological characterization of 5-HT_4 receptors (Clarke et al., 1989) and prompted a number of pharmaceutical companies to develop selective ligands for this receptor.

The first class of 5-HT_4 receptor to be discussed includes indole derivatives such as tryptamines (including 5-HT) and indole carbazimidamines derivatives. Tegaserod, prescribed to treat IBS in women suffering from constipation, belongs to this class. This compound was withdrawn from the market in 2007 because of cardiovascular side effects but has made a recent return to the market, albeit with limitations on use.

The second class includes benzamide derivatives such as metoclopramide, which was commercialized as an antiemetic (action via dopamine D_2 receptors and 5-HT_3 receptors at higher doses) and stimulating gastrointestinal transit (action via 5-HT_4 receptors). Mosapride, which also acts as a 5-HT_4 receptor agonist, is used to treat gastrointestinal disorders, as it accelerates gastric emptying (Curran and Robinson, 2008; Tack et al., 2012), but a main metabolite is pharmacologically active and antagonizes the 5-HT_3 receptor.

The benzofurane carboxamide prucalopride is selective for the 5-HT_4 receptor (Briejer et al., 1999) and has been approved for the treatment of chronic constipation, in case of laxative resistance, in Europe (2009) and in Canada (2011) (Tack et al., 2013).

Lead of the benzoates class is ML10302, which presents a high affinity for 5-HT_4 receptor and weak affinity for 5-HT_3 receptors (Yang et al., 1997). ML10302 was described as the first potent and selective 5-HT_4 receptor agonist, but further investigation in vivo was limited by its susceptibility to hydrolysis.

Benzodioxane class is represented by only one product, the SL65.0155, particularly proactive as a mnemonic in several species. This compound has reached Phase IIb for the treatment of Alzheimer disease (Moser et al., 2002). It was abandoned for undisclosed reasons that may be related to its low efficacy and very partial agonist profile on 5-HT_4 receptors.

The sixth class includes aryl ketones, which were developed to overcome the metabolic lability related to the related 5-HT_4 receptor ester ligands. These compounds have a high bioavailability and easily pass the blood-brain barrier. RS 67333 (Eglen et al., 1995a), containing a large alkyl group, was widely used in vivo in rodent and particularly for behavioral studies demonstrating the procognitive effects of 5-HT_4 receptor agonists (Fontana et al., 1997; Marchetti et al., 2000; Lamirault and Simon, 2001; Lelong et al., 2001; Kulla and Manahan-Vaughan, 2002).

The seventh class is represented by a carboxamide pyridine, PRX-03140 (also known in the literature as VRX-03011), another partial 5-HT_4 receptor agonist and promnemonic substance that reached Phase IIb for the treatment of Alzheimer disease (Mohler et al., 2007) in 2008, although development of the drug was then stopped.

Benzimidazolone derivatives constitute the eighth class with BIMU1 and BIMU8 as examples, which are potent and efficacious 5-HT_4 receptor agonists with good brain-penetrating properties (Dumuis et al., 1991; Rizzi et al., 1992). However, their affinity for 5-HT_3 and σ_2 receptors limits their utility (Bonhaus et al., 1993).

The last two classes include naphthalimide derivatives (Eglen et al., 1994) (RS 56532 and RS 66332) and the quinoline derivative velusetrag (Beattie et al., 2008). Velusetrag displays promising efficacy in patients with constipation (e.g., Nelson et al., 2017) and may also be useful in Alzheimer disease.

Other 5-HT_4 receptor agonists are listed in Table 16, indicating key pharmacological parameters.

2. Antagonists. GR 113808, belonging to the indole class, was the first selective 5-HT_4 receptor antagonist presenting low affinity for 5-HT_3 receptors (Gale et al., 1994) and the first tritiated radioligand commercially available. This molecule enabled the accurate definition of pharmacological characteristics and localization of 5-HT_4 receptors, especially in the brain (Grossman et al., 1993). Other antagonists are benzoate derivatives (such as SDZ 205557) or benzoate dioxane derivatives such as SB 204070, which by substitution of the chlorine by a radioactive iodine, lead to [^{125}I]SB 207710, another excellent radioligand (Kaumann et al., 1995). Other antagonist classes include benzimidazolones, imidazolpyridines, and aryl ketones.

5-HT_4 receptor antagonists have been mainly developed for the purpose of binding studies and in situ labeling using radioligands. Recent work includes the development of imaging probes for positron emission tomography (PET) (Dubost et al., 2012; Buiter et al., 2013; Tavares et al., 2014). However, because of the presence of 5-HT_4 in the failing heart, some antagonists have been designed for cardioprotective purposes, trying to avoid CNS penetration and improving oral administration route (Brudeli et al., 2010, 2013a,b, 2014).

Some 5-HT_4 receptor antagonists are listed in Table 16, indicating key pharmacological parameters.

3. Inverse Agonists. Inverse agonists of the 5-HT_4 receptor (Claeysen et al., 2000; Joubert et al., 2002) have

TABLE 16
Affinities and efficacies of several 5-HT_4 receptor ligands in radioligand binding (pK_i) and functional (pEC_{50}) studies

Compounds	Affinity	Efficacy		References
	pK_i	pEC_{50} or pIC_{50}	E_{max} or I_{max}	
Agonists				
PF-00885706	8.4	8.2–8.4	78%–84%	Komada et al., 2009
PF-01354082	8.7	8.1–8.5	74%–66%	Mikami et al., 2009
Compound 26	7.9	9.4	63%	McKinnell et al., 2009
ATI-75025 (Naronapride)	8.9			Camilleri et al., 2007; Bowersox et al., 2011
Compound 2d	9.3–9.8	9.2–9.6	7%–33%	Brodney et al., 2012
Compound 3	8.1–8.6	8.7–9.1	21%–63%	Brodney et al., 2012
TD-2749	8.0	8.6	85%	Long et al., 2012
PF-4995274 (TBPT)	9.5	9.0	19%	Sawant-Basak et al., 2013
SSP-002392	9.1–9.2	10.4–10.8	~100%	Tesseur et al., 2013
TD-8954	9.4	8.6	55%	McKinnell et al., 2013
Dual ligands				
5-HT_4 agonist/5-HT_3 antagonist Cpd 17	6.7 (5-HT_4R) 7.4 (H_3R)	7.3 (H_3R)		Lepailleur et al., 2014
Donecopride 5-HT_4 agonist/AChE inhibitor	8.1 (5-HT_4)	9.0 (5-HT_4) 7.8 (pIC_{50} AChE)	48% (5-HT_4)	Lecoutey et al., 2014; Rochais et al., 2015
Antagonists				
Compound 34d	8.8	9.2	80%–100%	Lemaître et al., 2009
Compound 30	8.8	7.9 (pKb)		Brudeli et al., 2010
Compound 12g	8.7	8.6	100%	Furlotti et al., 2012
Compound 20	11.9	10.6 (pKb)		Brudeli et al., 2013a
Compound 9	9.9	8.6 (pKb)		Brudeli et al., 2013b
Compound 25	10.1	9.1 (pKb)		Brudeli et al., 2014

been generated by Roche in collaboration with Bockaert's group (RO 116-0086, RO 116-1148, and RO 116-2617; Fig. 20). SB 207266, which is a carboxylate indole derivative, is also an inverse agonist at the 5-HT_4 receptor (Fig. 20).

4. Multifunctional Ligand. Dallemagne and colleagues reported the synthesis and characterization of donecopride, a multitarget directed molecule combining 5-HT_4 receptor agonist properties with acetylcholinesterase (AChE) inhibition (Lecoutey et al., 2014); the drug may display disease-modifying actions by increasing soluble amyloid precursor protein (sAPPα) release as well as procognitive effects (Lecoutey et al., 2014; Rochais et al., 2015).

5. 5-HT_4 Receptor Radioligands. The first tritiated or iodinated 5-HT_4 receptor compounds, mainly antagonists, were particularly useful to determine brain regional distribution of the receptor (Grossman et al., 1993; Waeber et al., 1993, 1994, 1996), [^{3}H]GR 113808 and [^{125}I]SB 207710 being the lead compounds (Kaumann et al., 1995). Then, several [^{11}C]-labeled compounds have been developed for use in PET (Gee et al., 2008; Xu et al., 2010; Buiter et al., 2013), enabling live imaging studies in animals (Kornum et al., 2009) and in humans (Marner et al., 2009; Madsen et al., 2011). New [^{125}I]-labeled antagonists have also been developed for use as PET tracers (Dubost et al., 2012). More recently, promising fluoride radioligands have been described: [^{18}F]MNI-698 and [^{18}F]MNI-699 (Caille et al., 2013; Tavares et al., 2014).

F. Function

1. Physiologic Function. The 5-HT_4 receptors have many physiologic functions. They are implicated in learning and memory and in the stimulation of the nonamyloidogenic cleavage of APP (Bockaert et al., 2008, 2011; Claeysen et al., 2015). Thus, they are potential targets to treat Alzheimer disease.

A role of 5-HT_4 receptors in feeding behavior is now clear. Agonists and antagonists have hypo- and hyperphagic properties, respectively (Jean et al., 2007; Bockaert et al., 2011).

5-HT_4 receptor stimulation has rapid antidepressant action and may contribute to the actions of SSRI (Lucas, 2009; Samuels et al., 2016).

In the periphery, the role of 5-HT_4 receptors in gastrointestinal tract is well established. 5-HT_4 receptors evoke gastric emptying, peristaltic reflex, intestinal motility, inhibition of hypersensibility, short free fatty acid–stimulated HCO_3^- secretion, and, importantly, enteric nervous system development and adult neurogenesis of enteric plexus (Matsuyoshi et al., 2010; Gershon, 2011, 2013; Hoffman et al., 2012; Akiba et al., 2015). For further discussion, see *XX. 5-HT Receptors and the Gastrointestinal Tract*.

Although it was initially believed that 5-HT_4 receptors were confined to the atria of porcine and human heart (Kaumann and Levy, 2006), functional 5-HT_4 receptors mediating increased cardiac contractility (inotropic effect), as well as hastened relaxation (lusitropic effect), were later found in porcine and human ventricle (Brattelid et al., 2004b). In human, and also rat ventricle, 5-HT_4 receptor mRNA expression and function is increased or even induced in pathologies such as heart failure and hypertrophy, and following cardiac infarction (Brattelid et al., 2004b; Qvigstad et al., 2005a,c), maybe reflecting reactivation of foetal gene expression (Brattelid et al., 2012). 5-HT_4 receptor antagonists may be useful in those cardiac pathologies (Birkeland et al., 2007a; Kjekshus et al., 2009).

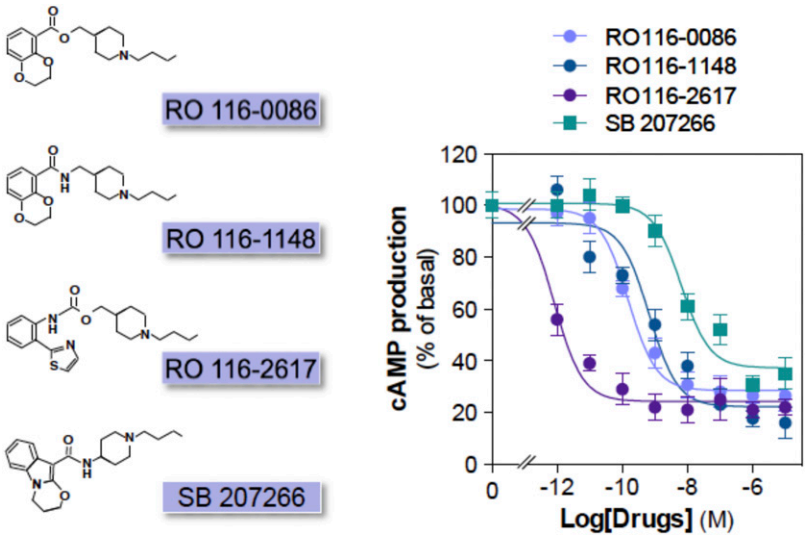

Fig. 20. Structure and activities of 5-HT_4 receptor inverse agonists. The inverse agonist activity was studied according to the ability of the compounds to inhibit the constitutive activity (activity in the absence of agonists). 5-$HT_{4(a)}$ receptors were expressed in COS-7 cells (1500 ± 130 fmol/mg). The constitutive cAMP production in presence of the receptor is equal to 720% ± 50% of the activity obtained in the absence of receptor. From Joubert et al. (2002).

Although 5-HT_4 receptors are weakly expressed in whole lung, airways epithelial cells, and airways smooth muscle cells, meta-analyses of genome-wide association studies indicate that intronic SNPs in *HTR4* gene are associated with some pulmonary diseases (Hancock et al., 2010; Repapi et al., 2010; Soler Artigas et al., 2011; Hodge et al., 2013).

5-HT_4 receptors are present in corticoadrenal gland where they stimulate aldosterone production, in salivary glands where they modify the volume and protein content of saliva (Turner et al., 1996; Bourdon et al., 2000), and in urinary bladder (Hegde and Eglen, 1996; Lefebvre et al., 2015).

2. Cellular Function.

a. G protein coupling of 5-HT_4 receptors and primary signaling events. 5-HT_4 receptors stimulate adenylyl cyclase in colliculi neurons as well as in hippocampus (Dumuis et al., 1988). This coupling has also been found in nucleus accumbens (Jean et al., 2007) and in heart atrium (Kaumann et al., 1990; Ouadid et al., 1992) and ventricle (Afzal et al., 2008). Thus, in native tissues, the 5-HT_4 receptor–mediated G_s/cAMP/PKA signaling pathway is well established. In primary cortical neurons and HEK293 cells, cAMP produced by 5-HT_4 receptors also activates the exchange factor Epac. Epac, via an Epac/Rap1/Ras pathway, activates an α-secretase and the release of sAPPα (Lezoualc'h and Robert, 2003; Cochet et al., 2013).

In transfected cell lines, several signaling pathways have been found, including coupling to $G_i/_o$, G_q, and G_{13} (Ponimaskin et al., 2002b; Bockaert et al., 2006; Woehler and Ponimaskin, 2009). Expression of different 5-HT_4 receptor splices variants in heterologous cells (COS and HEK293 cells, rodent cardiac myocytes) have identified putative differences in their signaling coupling. One clear difference is their constitutive activities measured on G_s coupling. Generally, the shorter the C terminus, the higher the constitutive activity (Claeysen et al., 1999). In addition, it has been reported that, in HEK293 cells and adult cardiac myocytes, 5-$HT_{4(b)}$ but not 5-$HT_{4(a)}$ or 5-$HT_{4(d)}$ are coupled to both G_s and $G_i/_o$ (Pindon et al., 2002). In similar heterologous systems, activation of transiently expressed 5-$HT_{4(a)}$ receptors by 5-HT and other agonists induce both cAMP and inositol phosphate accumulation (Chang et al., 2007; Gaven et al., 2013).

Following PKA activation, a series of ionic currents are modulated in colliculi and CA1 hippocampal neurons (Andrade and Chaput, 1991; Ansanay et al., 1995). These include a long-lasting inhibition of K^+ currents, including Ca^{2+}-activated K^+ channels (mediated by a PKA-dependent inhibition of phosphatases), which generates neuronal excitability and a decrease in spike accommodation (Ansanay et al., 1995) as well as activation of the hyperpolarization-activated current known to adjust repetitive discharge behavior in CA1 neurons (Andrade and Chaput, 1991). In prefrontal cortex pyramidal neurons, $GABA_A$ current and GABAergic transmission are either stimulated or inhibited by 5-HT_4 receptors. A role of PKA and A kinase anchoring proteins has been reported (Cai et al., 2002). 5-HT_4 receptors also activate L-type Ca^{2+} channels in human and porcine atrial myocytes via a cAMP/PKA pathway (Ouadid et al., 1992; Kaumann and Levy, 2006).

The receptor-G_s uncoupling phase of 5-HT_4 receptor desensitization is very rapid and efficient in colliculi neurons (Ansanay et al., 1992) and rat esophagus (Ronde et al., 1995) and is specifically dependent on the presence of a high expression of GRK2 but not of its catalytic activity (Barthet et al., 2005). In contrast, the β-arrestin/dynamin–dependent endocytosis of 5-HT_4 receptors in HEK293 cells does not require a high expression of GRK2 and is dependent of the S/T cluster upstream of L^{258} (Barthet et al., 2005).

b. G protein–independent coupling of 5-HT_4 receptors. 5-HT_4 receptors activate ERK in a G protein– and β-arrestin–independent manner, both in colliculi neurons and HEK293 cells (Barthet et al., 2007). The first step in 5-HT_4 receptor–ERK activation is the phosphorylation-activation of the tyrosine kinase Src bound to the receptor (Barthet et al., 2007). This G protein–independent activation of Src by 5-HT_4 receptors has also been described in epithelial cell lines (Gill et al., 2005). Indeed, in the enterocyte cell line, Caco 2, 5-HT_4 receptor–induced Src activation was required to activate the PLCγ1 pathway and to inhibit Na^+/H^+ exchanger (Gill et al., 2005).

5-HT_4 receptor–operated Src/ERK pathway, but not the G_s pathway, was negatively regulated by GRK5 physically preassociated with the proximal C-terminal region of the receptor in both human HEK293 cells and mouse colliculi neurons (Barthet et al., 2009). This desensitization requires two sequences of events: the association of β-arrestin1 to the phosphorylated S/T cluster already described and the phosphorylation, by GRK5, of β-arrestin1 (at S^{412}) bound to the receptor. Phosphorylated β-arrestin1, in turn, prevented the activation of Src constitutively bound to 5-HT_4 receptors (Barthet et al., 2009).

3. Gastrointestinal Tract. 5-HT_4 receptor localization and functions in GI tract have been extensively studied in many species, including guinea pig, pig, dog, mice, rat, and human (Craig and Clarke, 1990; McLean et al., 1995; Hegde and Eglen, 1996; Poole et al., 2006; Gershon and Tack, 2007; Sanger, 2008; Priem et al., 2013).

Activation of 5-HT_4 receptors is prokinetic on GI and stimulate guinea pig ileum contractions is concomitant to discovery of 5-HT_4 receptors (Craig and Clarke, 1990). Subsequently, 5-HT_4 receptor facilitation of the peristaltic reflex has been identified (Hegde and Eglen, 1996; Grider et al., 1998). The mechanism by which 5-HT_4 receptors stimulate peristaltic reflex is debated. Two proposals have been provided. 1) Within circular muscles of colon (human), 5-HT_4 receptors stimulate the release the inhibitory (descending relaxation) and excitatory (ascending contractions) neurotransmitters

(nitric oxide and acetylcholine respectively) (Tonini et al., 1989; Liu et al., 2005; Cellek et al., 2006; Ren et al., 2008). 2) 5-HT_4 receptors localized on the luminal surface of epithelial cells, including enterochromaffin cells and goblet cells, stimulate the peristaltic effect via secreted mucus, 5-HT, and fluid secretion (Hegde and Eglen, 1996). Luminal HCO_3^- secretion is an important component of normal colonic fluid and electrolyte movement and is a major fraction of the fluid secreted in several diarrheal diseases. It has been recently found that short-chain fatty acids (SCFAs) stimulate HCO_3^- secretion. This effect involves several steps, including the following ones. SCFA via the stimulation of a GPCR receptor localized on enterochromaffin cells (free fatty acid 2) increases 5-HT release, and 5-HT_4 receptors localized on epithelial cells and afferent neurons stimulate HCO_3^- secretion (Akiba et al., 2015).

4. Visceral Pain. Tegaserod, a 5-HT_4 receptor agonist, administered orally in human reduces abdominal pain and discomfort (De Maeyer et al., 2008b; Sanger, 2008). Because tegaserod is also a 5-HT_{2B} receptor antagonist, its exact mechanism of action is not clear. Hoffman et al. (2012) found that on a colorectal distension model of visceral pain, a more selective 5-HT_4 receptor agonist (naronapride) is also active and that both tegaserod and naronapride effects were blocked by a 5-HT_4 receptor antagonist. The mechanism of this antivisceral pain action of 5-HT_4 receptor agonists is unknown.

5. Enteric Nervous System Development and Enteric Nervous System Adult Neurogenesis. 5-HT_4 receptors are important in the early development of enteric neurons, whose number increases through 4 months of age (Gershon, 2013). In mice lacking 5-HT_4 receptors (KO), the early increase fails, and the later decline is more severe. 5-HT_4 receptors specifically protect cultured enteric neurons from apoptosis and also activate CREB (Liu et al., 2009). In adults, 5-HT_4 receptors are also able to stimulate neurogenesis in mice (Gershon, 2011). Adult neurogenesis appears to only start when ENS is injured, either following rectal transection and end-to-end anastomosis or chemical ablation with, for example, benzalkonium chloride (Matsuyoshi et al., 2010; Laranjeira et al., 2011). In the former model, mosapride, a 5-HT_4 receptor agonist, promoted the regeneration of the neural circuit in the impaired myenteric plexus and the recovery of the defecation reflex in the distal gut (Matsuyoshi et al., 2010; Kawahara et al., 2012).

6. Central Nervous System.

a. Learning and memory. One of the first roles of 5-HT_4 receptors in vivo was their promnesic effect in rat, mouse, monkey, and human (Eglen et al., 1995b; Bockaert et al., 2008, 2011; Haahr et al., 2013; Meneses, 2015). Procognitive effects of 5-HT_4 receptor agonists have been described, both on short-term memory (such as social olfactory memory) and on long-term olfactory memory (such as olfactory associative memory). Acute treatments with 5-HT_4 receptor agonists induced an increase in memory acquisition in autoshaping task, object and social recognition, Morris water-maze, task with long intertrial intervals (2 hours), delayed matching performance, and impeded spontaneous alteration scores (King et al., 2008; Bockaert et al., 2011). Chronic treatments also improve memory performance (Quiedeville et al., 2015) and increase working memory. Old rats have poor memory relative to adult rats that can be upgraded with 5-HT_4 receptor agonists, which are also reported to antagonize atropine- or scopolamine-induced learning and memory deficits in a variety of tasks, such as spatial navigation in Morris water maze, spontaneous alternation, and olfaction (Lamirault and Simon, 2001; Lelong et al., 2003; King et al., 2008; Marchetti et al., 2011). In 5-HT_4 receptor KO mice, the loss of learning and memory appears to be circumvented by adaptative changes in cholinergic systems (Buhot et al., 2003).

Hippocampal 5-HT_4 receptor expression correlates inversely with human memory (Haahr et al., 2013). The cellular basis of such learning and memory effects may be an increase in acetylcholine release found in frontal cortex and hippocampus, a complex modulation of synaptic plasticity (long-term potentiation and LTD) and a potentiation of learning-induced spine growth in the hippocampus [see Kemp and Manahan-Vaughan (2004), King et al. (2008), and Bockaert et al. (2011)]. Interestingly, the 5-HT_4 receptor agonist SL65.0155 enhances simultaneous olfactory discrimination performance and potentiates learning-induced dendritic spine growth in the mouse hippocampus (Restivo et al., 2008). Finally, an association between 5-HT_4 receptor mRNA and protein expression in cortical areas, hippocampus, olfactory tubercles on one hand, and memory consolidation on the other hand has been reported (Manuel-Apolinar et al., 2005).

b. Control of mood and role of 5-HT_4 receptor agonists. SSRIs are common drugs to treat depression that eventually increase tonic 5-HT release in relevant limbic structures such as the hippocampus. It has been established that stimulation of 5-HT_4 receptors localized on pyramidal mPFC stimulate the activity of a fraction of DRN 5-HT neurons (responder neurons with high-frequency discharges), an effect expected to result in a positive effect on mood behavior (Lucas and Debonnel, 2002; Lucas, 2009). Thus, 5-HT_4 receptor agonists may have relatively fast antidepressant actions or may be used in combination to reduce the delay of action of SSRIs when given alone (Samuels et al., 2016).

5-HT_4 receptors interact with p11, a protein that is downregulated in brain of depressed patients. This interaction is particularly clear in brain regions important for anxiety/depression and cognition such as the hippocampus (Warner-Schmidt et al., 2009; Egeland et al., 2011). The antidepressant-like activity of RS 67333, a 5-HT_4 receptor agonist, was abolished in p11-KO mice, which are known to have a depressive-like phenotype (Warner-Schmidt et al., 2009).

Adult hippocampal neurogenesis in the subgranular zone has gained considerable attention in relation to mood and depression. The hypothesis is that a decrease in newborn dentate granule cell production leads to depression, whereas enhanced neurogenesis (proliferation, survival, and maturation) is required for treatment of depression (Duman and Monteggia, 2006; Samuels et al., 2016). Chronic antidepressant treatments, including SSRIs, stimulate adult hippocampal neurogenesis (proliferation of newborn cells as well as the survival and maturation of the young neurons). 5-HT_4 receptor agonists can also induce a rapid neurogenesis in the hippocampus in adult rodents (Duman and Monteggia, 2006; Pascual-Brazo et al., 2012; Ishizuka et al., 2014) and the synthesis of BDNF, a key growth factor implicated in antidepressant actions and neurogenesis. Interestingly, the 5-HT_4 receptor antagonist GR 125487 partially blocks the neurogenic effects of chronic fluoxetine treatment and its antidepressant and anxiolytic effects (Mendez-David et al., 2014), which may relate to a reduced hippocampal 5-HT release evident with 5-HT_4 receptor antagonists (Ge and Barnes, 1996). In addition to neurogenesis, SSRIs can reverse neuronal maturation in the adult hippocampal granular cell neurons via 5-HT_4 receptor–mediated signaling (Kobayashi et al., 2010).

c. Feeding behavior. In 5-HT_4 receptor KO mice, the stress-induced hypophagia and novelty-induced exploratory activity were reduced (Compan et al., 2004). This suggests that 5-HT_4 receptors may be involved in stress-induced anorexia. Anorexia and bulimia are motivation disorders, which may implicate a reward structure such as nucleus accumbens that displays relatively high expression of 5-HT_4 receptors. Direct stimulation of 5-HT_4 receptors in the nucleus accumbens reduced the physiologic drive to eat and increased the mRNA of the anorectic peptide cocaine- and amphetamine-regulated transcript in WT but not KO mice (Jean et al., 2007, 2012). 5-HT_4 receptors control cocaine- and amphetamine-regulated transcript mRNA expression via a cAMP/PKA signaling pathway. Finally, intra-accumbal injection of 5-HT_4 receptor antagonists or siRNA-mediated 5-HT_4 receptor KO decrease satiety (Jean et al., 2007).

d. Cardiac system. 5-HT via 5-HT_4 receptors induces cardioexcitation in atrial but not in ventricles of healthy humans and pig (Kaumann, 1990; Kaumann et al., 1991; Jahnel et al., 1992). However, an inotropic effect can also be found in human and porcine cardiac ventricle under phosphodiesterase inhibition (Brattelid et al., 2004b). In the atrium, 5-HT_4 receptors activate and phosphorylate L-type Ca^{2+} channels via a cAMP/PKA signaling pathway (Ouadid et al., 1992; Kaumann and Levy, 2006). It has been proposed that atrial fibrillation, mediated by 5-HT_4 receptors, may occur in case of altered circulation during, for example, ageing (Kaumann and Levy, 2006). 5-HT_4 receptors expressed in sinoatrial node in piglets, and possibly in humans, may be responsible for some 5-HT–mediated arrhythmias (Sanders and Kaumann, 1992). In addition, in failing human heart (Brattelid et al., 2004b), there is an upregulation of 5-HT_4 receptor mRNA level in ventricles accompanied by a 5-HT_4 receptor–mediated positive inotropic response to 5-HT. Similar observations have been reported in infarcted, failing, and hypertrophic rat hearts (Qvigstad et al., 2005a,c; Brattelid et al., 2007a). Note that 5-HT_4 receptors are not expressed in healthy rat ventricles. Because in those pathologies there is an increase in plasma 5-HT concentration, likely released from platelets, this may result in cardiotoxic effects mediated via 5-HT_4 receptors.

Cardiac remodeling in heart failure is characterized by activation of a fetal gene program. Indeed, Brattelid et al. (2012) found, in rat, that 5-HT_4 receptor mRNA expression and 5-HT_4 receptor–mediated inotropic response are augmented not only in heart failure but also in ventricles during late fetal development. In rodent cardiomyocytes, prucalopride responses, characterized by a high propensity to trigger diastolic Ca^{2+} events, absent in controls, can be achieved following BDNF and imipramine treatments (Meschin et al., 2015). Because both treatments increase expression of p11, the authors are logically proposing that it is p11 that upregulates 5-HT_4 receptor responses as seen in the brain. This is likely but not formally demonstrated in the report. The difference in p11 expression in human and rodent cardiomyocytes could explain their different responsiveness to 5-HT_4 receptor agonists. This remains to be demonstrated.

For further discussion, see *XIX. 5-HT Receptors and the Cardiovascular System*.

e. Respiratory system. At the level of the brainstem central respiratory center, the Pre-Boetzinger complex, 5-$HT_{4(a)}$ receptors via a cAMP signaling pathway, control respiration (Manzke et al., 2003). They stimulate phrenic nerve activity and respiratory minute volume. Thus, 5-HT_4 receptors are able to avert opioid-induced breathing depression, likely by having an opposing effect on the cAMP decrease induced by those drugs in the respiratory center (Manzke et al., 2003). Although noncoding variant SNPs in *HTR4* are associated with pulmonary diseases in human genome?wide association studies (Hancock et al., 2010; Repapi et al., 2010; Soler Artigas et al., 2011; Hodge et al., 2013), the relatively low expression of 5-HT_4 receptors in lungs cast some doubt on the functional impact of 5-HT_4 receptors in pulmonary functions (Hodge et al., 2013). However, 5-HT_4 receptor KO mice display an altered baseline lung function (lung resistance, tissue resistance, and tissue elastance) and an increase in methacholine and 5-HT–induced airway hyper-responsiveness (House et al., 2015).

f. Adrenal gland. In the human adrenal gland, 5-HT_4 receptors are mainly localized on zona glomerulosa (Lefebvre et al., 2015), which is consistent with 5-HT stimulating preferentially aldosterone secretion

rather than cortisol in vitro. Similarly, 5-HT_4 receptor agonists administered to healthy individuals increases plasma aldosterone levels without any change in plasma cortisol concentrations (Lefebvre et al., 2015).

g. Urinary bladder. Neuromuscular cholinergic transmission in human isolated detrusor muscle is facilitated by neural 5-HT_4 receptors (Tonini et al., 1994). In contrast, in urinary bladder strips from rhesus and Cynomolgus monkeys, experiments using direct electrical stimulation of bladder smooth muscle indicate that the 5-HT_4 receptors are located postjunctionally (Waikar et al., 1994).

XII. 5-HT_{5A} Receptors

A. Introduction

In the early 1990s, the genes encoding the human and rodent 5-HT_{5A} receptor genes were cloned and localized. Subsequent work in the mid-1990s revealed the primary protein structure, expression pattern, and cellular function of the receptor using in vitro preparations (Plassat et al., 1992; Erlander et al., 1993; Matthes et al., 1993; Rees et al., 1994). Yet, the function of the receptor in vivo remained elusive until the early 2000s. By generating the *Htr5A* receptor knockout mouse, Grailhe et al. (1999, 2001) were the first to provide evidence of 5-HT_{5A} receptor function in vivo. Subsequently, an ex vivo approach was used to characterize the electrophysiological effects of 5-HT_{5A} receptors in native mouse and rat cortical brain tissue and the consequences of its deletion (Goodfellow et al., 2012), which led to the Receptor Nomenclature Committee promoting to receptor status (i.e., 5-ht_{5a} to 5-HT_{5A} receptor). Though further investigations have been pursued in vivo (Curtin et al., 2013; Muñoz-Islas et al., 2014; Yamazaki et al., 2014, 2015), clinical examination has been severely limited by the lack of selective ligands.

B. Gene and Primary Structure

The human *HTR5A* is located on chromosome 7 at position 7q36 (Matthes et al., 1993). Partial sequencing revealed an intron-exon boundary located in the middle of the third cytoplasmic loop at exactly the same position as in the mouse and rat 5-HT_{5A} genes (Rees et al., 1994; Grailhe et al., 2001). The homologous mouse *Htr5A* is located on chromosome 5 at position 5B (Matthes et al., 1993). Partial sequence analysis revealed that the 5-HT_{5A} receptor gene contains an intron in the third cytoplasmic loop (Matthes et al., 1993), approximately 8 kb in length (Matthes et al., 1993). The human 5-HT_{5A} receptor, composed of a 1071-bp open reading frame, displays approximately 82% nucleotide homology with the mouse 5-HT_{5A} gene (Rees et al., 1994; Grailhe et al., 2001).

Polymorphisms in the 5-HT_{5A} receptor have been reported, including two nucleotide substitutions in the 5′ untranslated region and two synonymous substitutions in the coding region as well as a Pro15Ser amino acid substitution near the N terminus (Shimron-Abarbanell et al., 1997; Iwata et al., 1998; Birkett et al., 2000; Iwata et al., 2001; Arias et al., 2001; Dubertret et al., 2004). The latter polymorphism is proximal to a phosphorylation site and is located in a region likely involved in agonist-induced downregulation (Iwata et al., 1998; Thomas, 2006). Additional polymorphisms in the promoter region of the 5-HT_{5A} receptor have been identified (Zhang et al., 2010).

Hydropathy analysis revealed that the 5-HT_{5A} receptor contains seven hydrophobic domains (numbered I–VII), a classic feature of GPCRs (Plassat et al., 1992; Erlander et al., 1993; Matthes et al., 1993; Rees et al., 1994; Grailhe et al., 2001; Thomas et al., 2004). Sequencing analysis of the 5-HT_{5A} receptor protein uncovered a single long open reading frame and a poly A tail (Plassat et al., 1992; Matthes et al., 1993; Hurley et al., 1998; Grailhe et al., 2001; Thomas et al., 2004). The length of the 5-HT_{5A} receptor protein is 357 amino acids for the human, mouse, and rat proteins (Plassat et al., 1992; Erlander et al., 1993; Matthes et al., 1993; Rees et al., 1994; Hurley et al., 1998; Grailhe et al., 2001) but 356 amino acids in length for the guinea pig receptor (Thomas et al., 2004). In all species, the 5-HT_{5A} receptors display *N*-linked glycosylation sites on the amino terminals as well as consensus phosphorylation sites for protein kinase C and protein kinase A on the presumed cytoplasmic domains (Plassat et al., 1992; Erlander et al., 1993; Grailhe et al., 2001).

C. Expression Profile

The 5-HT_{5A} receptor is expressed widely in the central nervous system (Plassat et al., 1992; Rees et al., 1994; Pasqualetti et al., 1998a; Grailhe et al., 2001; Kinsey et al., 2001; Tanaka et al., 2012; Fig. 21) but seemingly not in peripheral organs (Plassat et al., 1992; Rees et al., 1994; Grailhe et al., 2001). There is some controversy over its expression in the peripheral nervous system (Pierce et al., 1996; Chen et al., 1998a; Wang et al., 2000; Nicholson et al., 2003; Avila-Rojas et al., 2015).

In the human brain, the 5-HT_{5A} receptor mRNA expression has been reported in many regions of the cerebral cortex, including prefrontal, parietal, temporal, occipital, and entorhinal cortices (Pasqualetti et al., 1998b; Grailhe et al., 2001; Lambe et al., 2011; Fig. 22). The 5-HT_{5A} mRNA was also detected in the hippocampus, the amygdala, the cerebellum, the striatum, the caudate nucleus, and the substantia nigra (Rees et al., 1994; Pasqualetti et al., 1998a; Grailhe et al., 2001; Fig. 22). Similar expression patterns have been reported in the mouse (Plassat et al., 1992; Tanaka et al., 2012) and the rat (Erlander et al., 1993; Kinsey et al., 2001; García-Alcocer et al., 2010; Lopez-Esparza et al., 2015). Expression was also noted in the suprachiasmatic nucleus of the hypothalamus, raphe nuclei, the ventral tegmental area,

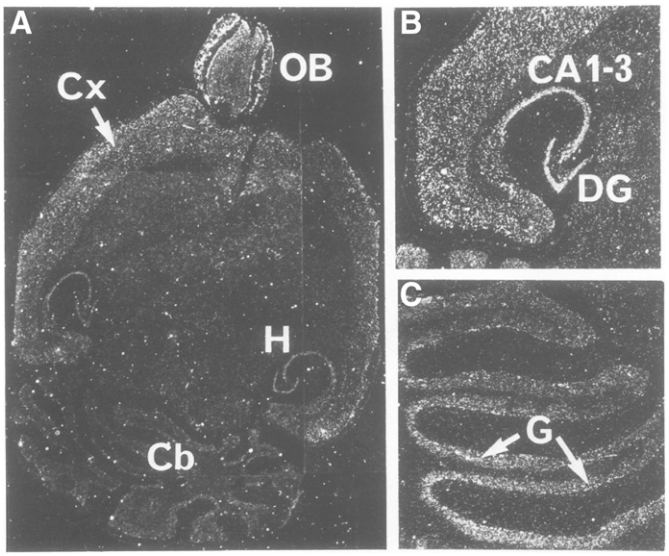

Fig. 21. In situ hybridization detection of 5-HT_{5A} receptor mRNA expression in mouse brain. (A) Dark field of the emulsion autoradiograph of a horizontal section through an adult mouse brain, with images at higher magnification showing (B) hippocampus and (C) cerebellum. CA1-3, CA fields of the hippocampus CA; Cb, cerebellum; Cx, cerebral cortex; DG, dentate gyrus; G, granule cell layer of the cerebellum; H, hippocampus; OB, olfactory bulb. Adapted from Plassat et al. (1992) (with permission).

and the locus coeruleus (Duncan et al., 2000; Oliver et al., 2000). Because the 5-HT_{5A} receptor mRNA has not been detected in astrocytes (Hirst et al., 1998), expression of this receptor in the central nervous system is likely restricted to neuronal cells, consistent with the morphology of cells expressing 5-HT_{5A} receptor immunoreactivity (Oliver et al., 2000).

D. Post-translational Modifications and Impact

Treatment with the *N*-glycosylation inhibitor tunicamycin fails to alter [^{3}H]5-HT binding to the 5-HT_{5A} receptor yet results in redistribution of the 5-HT_{5A} receptor from the cell membrane to the intracellular compartment, suggesting that the *N*-glycosylation sites may play a role in targeting the 5-HT_{5A} receptor to the cell membrane (Dutton et al., 2008). Although both the N6 and N21 residues appear *N*-glycosylated within the mature human receptor protein, amino acid mutagenesis demonstrates that *N*-glycosylation of only the N6 residue results in protein insertion into the cell membrane (Dutton et al., 2008), a presumed prerequisite for receptor function.

E. Pharmacology

The psychedelic hallucinogen LSD binds to and stimulates the 5-HT_{5A} receptor with high affinity, as does another nonselective 5-HT receptor agonist, 5-CT. However, the quest to characterize the functions of the 5-HT_{5A} receptor in native tissue has been limited by a lack of selective agonists. Such characterization has relied on nonselective agonists in the presence of antagonists for other 5-HT receptors (Goodfellow et al., 2012).

Among selective antagonists for 5-HT_{5A} receptors, SB699551 (Corbett et al., 2005) has been most widely used in the literature (Table 17). It is important to note, however, that although SB-699551 possesses high affinity for the human and guinea pig 5-HT_{5A} receptor (~pK_i 8.3), it displays a 100-fold lower affinity for the rat and mouse 5-HT_{5A} receptor (pK_i 6.3; Thomas et al., 2006), limiting its utility in preclinical studies.

Other 5-HT_{5A} receptor antagonists have been proposed, including A-842377 (Garcia-Ladona et al., 2006) and 2-aminodihydroquinazolines (Peters et al., 2008). However, only SB699551 and a series of compounds, ASP5736 (Yamazaki et al., 2014, 2015), AS2030680, and AS2674723 (Yamazaki et al., 2015) have extensive published pharmacological validation. Nonselective antagonists for the 5-HT_{5A} receptor include methiothepin and ritanserin (Thomas et al., 2004).

Based on rank order of affinity alone, the following in vitro pharmacological profile can be suggested for the human and mouse 5-HT_{5A} receptors: LSD (human pK_i 8.40–8.70; mouse pK_i 9.10) > 5-CT (human pK_i 7.6–8.28; mouse pK_i 7.8) > 5-HT (human pK_i 6.7–7.40; mouse pK_i 6.6–7.12) > 8-OH-DPAT (human pK_i 5.6–6.07; mouse pK_i 6.13–5.9) (Plassat et al., 1992; Rees et al., 1994; Francken et al., 1998; Grailhe et al., 2001; Thomas et al., 2004). By contrast, the human and mouse 5-HT_{5A} receptors display low affinity for ketanserin, norepinephrine, dopamine, or spiperone (pK_i < 5) (Plassat et al., 1992; Rees et al., 1994; Francken et al., 1998).

F. Function

1. Cellular Function. Functional effects of 5-HT_{5A} receptors have been examined in cell systems, in native tissue, and in vivo. Broadly, these data suggest that the 5-HT_{5A} receptor can activate pertussis toxin–sensitive G proteins (Francken et al., 1998; Hurley et al., 1998; Thomas et al., 2004) and suppress adenylyl cyclase and production of cAMP (Francken et al., 1998; Hurley et al., 1998; Thomas et al., 2000; Noda et al., 2003). Electrophysiological examination in native rodent cerebral cortex is consistent with coupling to $G\alpha_{i/o}$ proteins that activate Kir3 channels (Goodfellow et al., 2012). Studies in vivo indicate 5-HT_{5A} receptors may influence cognitive function and memory (Gonzalez et al., 2013; Yamazaki et al., 2014, 2015; Nikiforuk et al., 2016) and participate in acoustic startle circuitry (Curtin et al., 2013) and potentially antinociception (Muñoz-Islas et al., 2014).

2. G Protein Coupling of the 5-HT_{5A} Receptor In Vitro. To elaborate on the functional work in cell systems, treatment of cell lines transfected with the 5-HT_{5A} receptor with either 5-HT or 5-CT increases labeled GTPγS binding (Francken et al., 1998; Hurley et al., 1998; Thomas et al., 2004), an effect that could be abolished by pretreatment with pertussis toxin (Francken et al., 1998; Thomas et al., 2004). Conversely, pretreatment of the nonhydrolysable GTP analog, Gpp(NH)p,

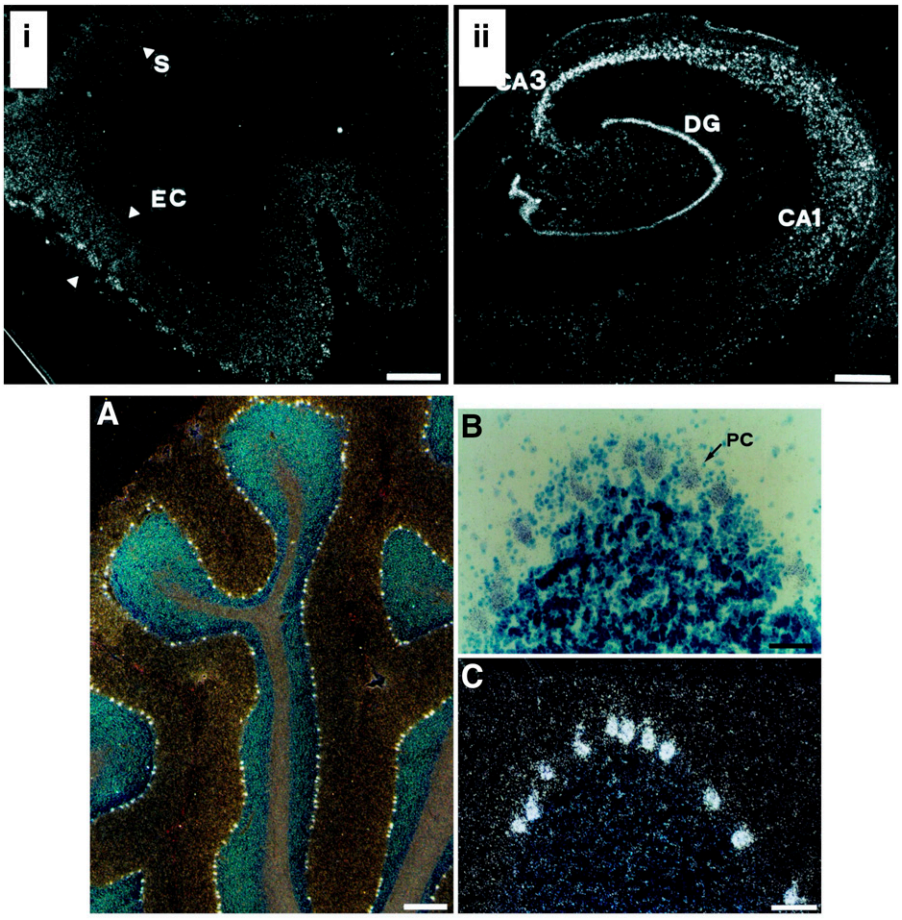

Fig. 22. In situ hybridization detection of 5-HT_{5A} receptor mRNA expression in human brain. (i and ii) Dark-field autoradiographs of coronal sections of human hippocampus and surrounding regions. CA1, CA3 fields and the dentate gyrus (DG) of hippocampus, entorhinal cortex (EC), and subiculum (S). Scale bars, 0.2 cm. (A) Dark-field autoradiograph of a coronal section of the cerebellar cortex: the Purkinje cells are heavily labeled [high magnification of Purkinje cells in bright-field (B) and dark-field (C)]. Scale bars, 600 μm (A), 500 μm (B), and 900 μm (C). Adapted from Pasqualetti et al. (1998b) (with permission).

reduced the high affinity binding of [^{3}H]5-CT in transfected HEK293 cells (Francken et al., 1998). Moreover, stimulation of the 5-HT_{5A} receptor with 5-HT can reduce the basal activity of adenylyl cyclase (Noda et al., 2003) and inhibit forskolin-induced formation of cAMP in both C6 glioma and HEK293 cell lines (Francken et al., 1998;

TABLE 17
Affinities of selective antagonists at 5-HT_{5A} and other 5-HT receptors

Adapted from data reported in Corbett et al. (2005) (for SB699551; compound 11a), Thomas (2006) (for SB699551), and Yamazaki et al. (2015) (for ASP5736, AS2030680, and AS2674723). Values are binding IC_{50} or K_i (where indicated). Note K_i values are given for the human, mouse, and rat cloned 5-HT_{5A} receptors, whereas the other values have been reported mainly for the human receptor (also see Thomas et al., 2004 for guinea pig values).

Receptor	Species	SB699551	ASP5736	AS2030680	AS2674723
5-HT_{1A}	Human	K_i = 501 nM	>1000 nM	K_i = 21 nM[a]	K_i = 84 nM
5-HT_{1B}	Human	K_i = 316 nM	NR	NR	NR
5-HT_{1D}	Human	K_i = 398 nM	NR	NR	NR
5-ht_{1e}	Human	K_i > 1000 nM	NR	NR	NR
5-HT_{1F}	Human	K_i > 1000 nM	NR	NR	NR
5-HT_{2A}	Human	K_i = 794 nM	>1000 nM	>300 nM	>300 nM
5-HT_{2B}	Human	K_i = 1000 nM	>1000 nM	K_i = 22 nM[a]	>300 nM
5-HT_{2C}	Human	K_i = 398 nM	K_i = 287 nM	>300 nM	>300 nM
5-HT_3	Human	NR	>1000 nM	>100 nM	>300 nM
5-HT_4	Guinea pig	NR	>1000 nM	>100 nM	>300 nM
5-HT_{5A}	Human	K_i = 3 - 6 nM[a]	K_i = 4 nM[a]	K_i = 1 nM[a]	K_i = 1 nM[a]
5-HT_{5A}	Rat	K_i = 501 nM	K_i = 2 nM[a]	K_i = 1 nM[a]	K_i = 1 nM[a]
5-HT_{5A}	Mouse	K_i = 501 nM	K_i = 4 nM[a]	K_i = 3 nM[a]	K_i = 2 nM[a]
5-HT_6	Human	K_i > 1000 nM	>1000 nM	K_i = 39 nM	>300 nM
5-HT_7	Human	K_i > 1000 nM	K_i = 123 nM	K_i = 10 nM[a]	K_i = 7 nM[a]

NR, not reported.
[a]High affinity binding at 5-HT_{5A} receptors or medium- to high-affinity nonselective binding.

Hurley et al., 1998; Thomas et al., 2000; Noda et al., 2003). Moreover, pretreatment with pertussis toxin prevented this 5-HT–elicited inhibition of basal adenylyl cyclase activity (Noda et al., 2003). These findings are also consistent with the 5-HT_{5A} receptor coupling to a Gαi/o protein pathway. However, this inhibitory effect of the 5-HT_{5A} receptor on cAMP could not be replicated in transfected HeLa or COS-M6 cells (Erlander et al., 1993). Similarly, in both HEK and NIH-3T3 cell lines, stimulation of the transfected 5-HT_{5A} receptors had no detectable effect in basal cAMP levels, forskolin-induced cAMP levels, or accumulation of inositol phosphates (Grailhe et al., 2001). In C6 glioma cells, 5-HT_{5A} receptor has been shown to inhibit ADP-ribosyl cylase activity in response to 5-HT, an effect that was abolished following pretreatment with pertussis toxin (Noda et al., 2003).

3. G Protein Coupling of the 5-HT_{5A} Receptor Ex Vivo. Research in native tissue has shown that cerebral cortical 5-HT_{5A} receptors couple to G proteins and has assessed the electrophysiological consequences of such coupling. In the presence of clozapine and spiperone, cortical slices from wild-type mice display a rightward shift of the [^{3}H]5-CT binding following pretreatment with Gpp(NH)p, suggesting that in the native system, the 5-HT_{5A} receptor is coupled to G proteins (Waeber et al., 1998). The 5-HT_{5A} receptor–activated ion current has been characterized in native prefrontal cortical tissue of both rats and mice (Goodfellow et al., 2012). The native 5-HT_{5A} receptor produces a small outward current that has a potent inhibitory effect on excitability of layer V pyramidal neurons of the prefrontal cortex. 5-HT has submicromolar potency at the native 5-HT_{5A} receptor and activates inwardly rectifying potassium channels (Goodfellow et al., 2012).

4. 5-HT_{5A} Receptor Function in the Spinal Cord. Spinal 5-HT_{5A} receptors are upregulated by painful stimuli (Muñoz-Islas et al., 2014) and have been suggested to participate in antinociception and pain (Doly et a., 2004; Muñoz-Islas et al., 2014; Avila-Rojas et al., 2015). Their spinal localization suggests possible motor, somatosensory, and autonomic functions (Doly et al., 2004).

5. Function In Vivo. Grailhe et al. (1999) generated 5-HT_{5A} receptor knockout mice. In terms of its biologic phenotype, the central nervous system of the 5-HT_{5A} receptor knockout mice appeared normal. There were no major differences in the cytoarchitectonic divisions, neuronal morphology, or distribution of glia (Grailhe et al., 1999). Moreover, 5-HT_{5A} receptor knockout mice display no differences in the distribution of neuronal markers for the monoaminergic system, calcium binding protein, neuropeptides, nitric oxide synathase, or amino acid receptor subunits (Grailhe et al., 1999).

There appears to be the potential for significant cross talk between 5-HT_{5A} receptors and 5-HT_{1A} receptors in cerebral cortex given their electrophysiological function (Goodfellow et al., 2012, 2014). In mice constitutively deleted for *Htr5A*, however, there were striking, supracompensatory changes in the magnitude of the prefrontal 5-HT_{1A} receptor currents, with 5-HT_{5A} receptor knockout mice displaying nearly a doubling of the 5-HT_{1A} receptor outward current in layer V neurons from the prefrontal cortex (Goodfellow et al., 2012). The mechanism underlying this homeostatic plasticity is unknown. Further electrophysiological examination of 5-HT_{5A} receptor knockout mice revealed no difference in $GABA_B$ receptor–mediated outward current, 5-HT_{1A} receptor protein levels, or cell intrinsic properties in the adult prefrontal cortical neurons (Goodfellow et al., 2012).

Mice deleted for *Htr5A* display an increase of explorative-like behaviors, rather than anxiety-like behaviors, on the open field, the elevated plus maze, and the marble burying task (Grailhe et al., 1999). Moreover, 5-HT_{5A} receptor knockout mice display greater increase in the number of entries into the central region after the introduction of the novel object into the center of the open field, suggesting an increase in "inspective" explorative-like behaviors (Grailhe et al., 1999). In addition, 5-HT_{5A} receptor knockout mice displayed a reduction on LSD-elicited increase in locomotion in the open field (Grailhe et al., 1999). These changes occurred in the absence of any difference in motor activity (Grailhe et al., 1999). Given the evident considerable homeostatic plasticity detected in the cerebral cortex of the 5-HT_{5A} receptor knockout mice (strong upregulation of 5-HT_{1A} receptor–mediated effects; Goodfellow et al., 2012), the behavioral characterization of the 5-HT_{5A} receptor knockout may underestimate its behavioral effects.

G. Clinical Relevance

The 5-HT_{5A} receptors are positioned neuroanatomically to play a role in emotional regulation, cognition, antinociception, and control of circadian rhythms and metabolism (Plassat et al., 1992; Pasqualetti et al., 1998a,b; Oliver et al., 2000; Duncan et al., 2000; Grailhe et al., 2001; Doly et al., 2004; Lambe et al., 2011; Tanaka et al., 2012). Unfortunately, clinical examination of 5-HT_{5A} receptors has been restricted by the lack of selective ligands.

1. Psychosis and Depression. A nonsynonomous SNP (Pro15Ser) in the *HTR5A* gene has been linked to schizophrenia (Iwata et al., 2001; Dubertret et al., 2004) and depression (Birkett et al., 2000), and polymorphisms in the promoter region of the 5-HT_{5A} receptor link to elevated triglyceride levels (Zhang et al., 2010). The high affinity of the psychedelic hallucinogen LSD for the 5-HT_{5A} receptor also underscores a potential link to psychosis (Thomas, 2006), keeping in mind that LSD, like many other ergolines, has affinity for multiple 5-HT receptors, of which only the 5-HT_{2A} receptor has been shown to play a significant role in hallucinations or psychosis. Yet in additional relevance to schizophrenia,

5-HT_{5A} receptors have been suggested to participate in prepulse inhibition of the acoustic startle response (Curtin et al., 2013). This effect was demonstrated in goldfish with SB-699551 and with A-843277. The latter compound has been reported to have antipsychotic and antidepressant properties in rodent models, in line with the distribution of the receptor in higher cortical and limbic regions (Garcia-Ladona et al., 2006; Jongen-Relo et al., 2006; but see Kassai et al., 2012). Recent work suggests that blocking 5-HT_{5A} receptors may ameliorate positive symptoms in a rodent model of schizophrenia and will potentially enhance cognition and social behavior in such models (Yamazaki et al., 2014; Nikiforuk et al., 2016). It may be relevant that the atypical antipsychotic drug asenapine has high affinity for h5-HT_{5A} receptors (along with a number of other receptors; Shahid et al., 2009).

2. Memory and Circadian Rhythm. Yamazaki et al. (2014, 2015) demonstrated the 5-HT_{5A} receptor antagonists (ASP5736, AS2030680, and AS2674723; Table 17) benefit memory, supporting a role for 5-HT_{5A} receptors in memory consolidation (Gonzalez et al., 2013). Additional preclinical work focuses on the localization of 5-HT_{5A} receptors in the hypothalamus, where it is densely expressed in the suprachiasmatic nucleus (Oliver et al., 2000; Duncan et al., 2000), suggesting potential roles in circadian rhythm and metabolism.

XIII. 5-ht_{5b} Receptors

A. Introduction

The genes encoding the human and rodent 5-ht_{5b} receptor were identified in 1993. The human 5-ht_{5b} receptor gene *HTR5B* is located on chromosome 2 at position 1q11–13 (Matthes et al., 1993). However, this gene contains stop codons in exon I, resulting in a transcriptional product likely yielding a presumed nonfunctional truncated protein (Grailhe et al., 2001). The mouse 5-ht_{5b} receptor gene *Htr5B* is located on chromosome 1 at position 1F (Matthes et al., 1993) and forms a functional protein upon heterologous expression. Partial sequence analysis revealed that the 5-ht_{5b} receptor gene contained an intron in the third cytoplasmic loop (Matthes et al., 1993; Grailhe et al., 2001).

B. Receptor Structure

Using hydropathy analysis, the predicted 5-ht_{5b} receptor protein has been shown to contain seven hydrophobic domains (numbered I–VII), a classic feature of GPCRs (Plassat et al., 1992, 1992; Erlander et al., 1993; Matthes et al., 1993; Wisden et al., 1993; Rees et al., 1994; Grailhe et al., 2001). Sequencing analysis of the 5-ht_{5b} receptor protein revealed a single long open reading frame and a poly A tail (Erlander et al., 1993; Matthes et al., 1993; Wisden et al., 1993). The length of the 5-ht_{5b} receptor is 370 amino acids in both the rat and mouse protein (Erlander et al., 1993; Matthes et al., 1993; Wisden et al., 1993).

C. Expression Profile

The 5-ht_{5b} receptor has a restricted expression profile within the central nervous system. In the rodent, expression of the 5-ht_{5b} receptor is exclusively found in the CA1 field of the hippocampus, the habenula, the inferior olivary nucleus, and the raphe nuclei (Erlander et al., 1993; Matthes et al., 1993; Wisden et al., 1993; Kinsey et al., 2001; Serrats et al., 2004, 2004; Tanaka et al., 2012; Fig. 23). Interestingly, the 5-ht_{5b} receptor mRNA was strongly expressed in the medial portion of the raphe nuclei and was found to have high coexpression with 5-HT transporter, suggesting that this receptor may be localized on 5-HT–producing neurons (Erlander et al., 1993; Matthes et al., 1993; Wisden et al., 1993; Serrats et al., 2004; Fig. 24). Expression of the 5-ht_{5b} receptor has not been detected in any peripheral organs, including the heart, kidney, lung, liver, or intestine (Erlander et al., 1993; Matthes et al., 1993; Wisden et al., 1993). Evidence for the 5-ht_{5b} receptor being expressed primarily in endosomes rather than the cell membrane raises potentially novel mechanisms of the functional relevance of this protein (Niebert et al., 2017) by impacting cell membrane expression of the 5-HT_{1A} receptor via intracellular direct protein-protein interaction.

D. Regulatory Mechanisms and Post-translational Modifications

The 5-ht_{5b} gene expression is regulated by the transcription factor ATF-7, which can directly bind to the 5-ht_{5b} promoter region, resulting in histone methylation and, ultimately, silencing of the receptor gene transcription (Maekawa et al., 2010). The 5-ht_{5b} receptor displays *N*-linked glycosylation sites on the amino-terminal end as well as consensus phosphorylation sites for protein kinase C and protein kinase A on the presumed cytoplasmic domains (Erlander et al., 1993; Matthes et al., 1993; Wisden et al., 1993).

E. Pharmacology

To date, selective ligands for the 5-ht_{5b} receptor have not been reported. Nonselective agonists include LSD and 5-CT, and a nonselective antagonist is methiothepin. Based on affinity alone, the following in vitro pharmacological profile can be suggested for rodent 5-ht_{5b} receptors: LSD (rat pKi 7.49), 5-CT (mouse pKi 7.4; rat pKi 6.26–8.88), and methiothepin (mouse pKi 7.8; rat pKi 7.35–8.87) but low affinity for ketanserin, dopamine, and norepinephrine (Matthes et al., 1993).

F. Function at Cellular, Tissue, and In Vivo Levels

The transduction pathway of the 5-ht_{5b} receptor has not been well examined. Pretreatment with Gpp(NH)p reduced [^{3}H]5-CT binding in 5-ht_{5b}–transfected COS1 cells, suggesting that the 5-ht_{5b} receptor readily couples

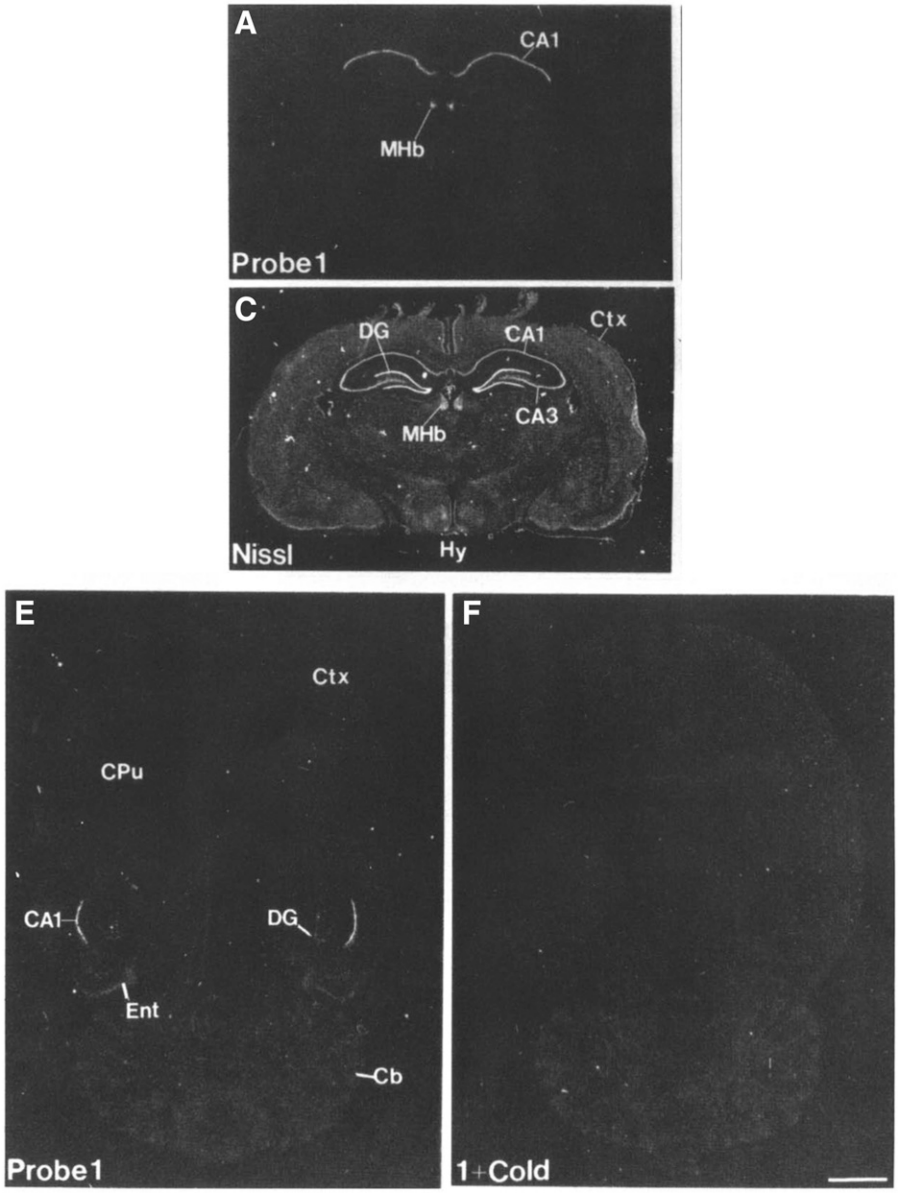

Fig. 23. In situ hybridization detection of 5-ht$_{5b}$ receptor mRNA expression in rat brain. Coronal (A) and horizontal (E) rat brain sections. (C) Nissl stain of the section used to generate the autoradiogram in (A). (F) Radioactive oligonucleotide probe in the presence of excess unlabeled probe. CA1 and CA3, CA fields of the hippocampus; Cb, cerebellum; CPu, caudate-putamen; Ctx, cortex; DG, dentate granule cells; Ent, entorhinal cortex; Hy, hypothalamus; MHb, medial habenula. Adapted from Wisden et al. (1993) (with permission).

to G protein (Wisden et al., 1993). However, this receptor had no effect on basal cAMP levels in either HeLa or COS-M6 cell lines (Erlander et al., 1993), although high levels of 5-ht$_{5b}$ expression in mouse brain are associated with low cAMP levels (Vogelgesang et al., 2017).

It is speculated that the 5-ht$_{5b}$ receptor acts as an inhibitory autoreceptor on 5-HT neurons in the raphe in rodents. However, neither an electrophysiological nor pharmacological profile has been described for the 5-ht$_{5b}$ receptor in native tissue.

In a mouse model of Rett syndrome, there is a relatively high level of 5-ht$_{5b}$ receptor expression in the brainstem because of a failure to downregulate receptor expression (Vogelgesang et al., 2017), with further studies suggesting a role for the 5-ht$_{5b}$ receptor in the modulation of the complex breathing phenotype of the mouse model of Rett syndrome (Vogelgesang et al., 2018) as well a potential link to the native receptor inhibiting generation of cAMP.

G. Clinical Relevance

Functional 5-ht$_{5b}$ receptors are likely not expressed in humans (Grailhe et al., 2001), which limits interest in this receptor particularly from the pharmaceutical industry.

XIV. 5-HT$_6$ Receptors

A. Introduction

The 5-HT$_6$ receptor has moderately high affinity for 5-HT, with an apparent binding affinity of approximately 30 nM. The gene for the 5-HT$_6$ receptor was

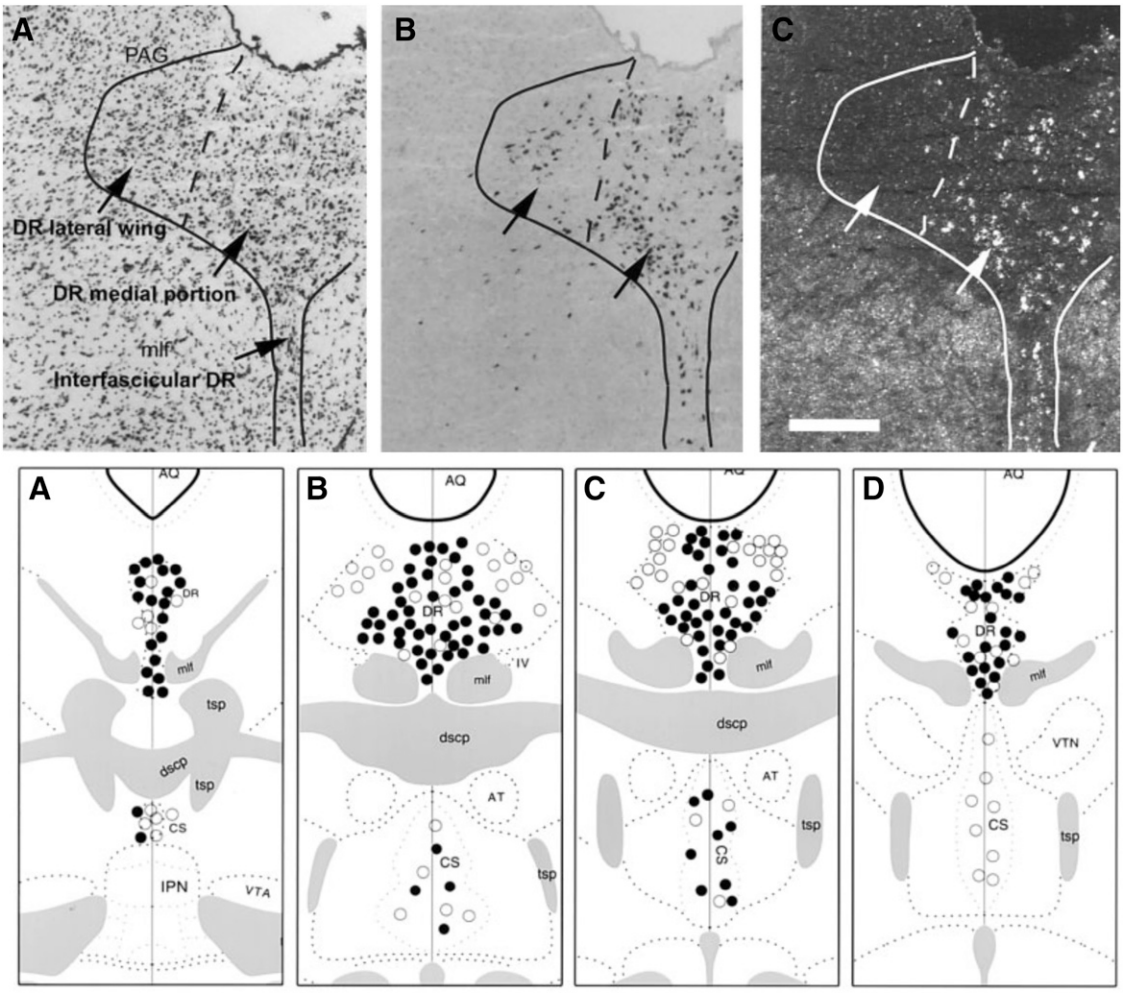

Fig. 24. Evidence for 5-HT raphe neurons expressing the 5-ht$_{5b}$ receptor. Top row: Visualization of 5-HTT and 5-ht$_{5b}$ receptor mRNAs in the dorsal raphe. (A) A consecutive section to (B and C), stained with cresyl violet for anatomic reference. The approximate limits of the dorsal raphe (DR) and its subdivisions—lateral wings and medial portion—are depicted. (B) a bright-field photomicrograph where numerous cell profiles express 5-HTT mRNA are visualized using digoxigenin-labeled oligonucleotide probes. (C) A dark-field photomicrograph from an emulsion-dipped tissue section displaying 5-ht$_{5b}$ receptor mRNA signal in the DR. mlf, medial longitudinal fasciculus; PAG, periaqueductal gray. Scale bar, 0.5 mm. Bottom row: Schematic representations of the rat lower midbrain and upper pons showing the subregional location of cells coexpressing 5-ht$_{5b}$ receptor mRNA and 5-HTT mRNA (filled circles) and cells expressing only 5-HTT mRNA (empty circles) in the DR and central superior nucleus (CS or median raphe). AQ, cerebral aqueduct; AT, anterior tegmental nucleus; dscp, decussation of the superior cerebellar peduncle; IPN, interpeduncular nucleus; tsp, tectospinal pathway; VTA, ventral tegmental area; VTN, ventral tegmental nucleus. Adapted from Serrats et al. (2004) (with permission).

originally cloned from rat in the early 1990s (Monsma et al., 1993; Ruat et al., 1993a) and later from human (Kohen et al., 1996) and mouse (Kohen et al., 2001) cDNA libraries; the human gene was located on chromosome 1 (Kohen et al., 1996). For each known species, the complete gene contains three exons and has a total length of around 14 kb with a coding sequence of approximately 1.4 kb. The receptor protein is composed of 440 amino acids in humans and mice and 436 amino acids in rats; a sequencing error in the originally published rat sequence was later corrected (Kohen et al., 1996). In humans, there is one described polymorphism (a C267T variant; Masellis et al., 2001) as well as a truncated, nonfunctional splice variant (Olsen et al., 1999). No replicated polymorphisms in the human gene have been associated with human diseases, including schizophrenia, methamphetamine-associated psychosis, tardive dyskinesia, Alzheimer disease, obesity, or antidepressant drug responsiveness.

The protein structure of the 5-HT$_6$ receptor conforms to the typical motif of GPCRs with the typical seven transmembrane domain configuration. There have been no detailed investigations of whether this receptor exists in dimers or higher-order oligomers.

B. Expression

5-HT$_6$ receptor mRNA is expressed primarily in the brain (Fig. 25), being detectable in rat brain by day 12 of embryogenesis (Grimaldi et al., 1998). Originally, Northern blot analyses detected RNA transcripts in brain tissue with the following regional density profile: hypothalamus > hippocampus = mesencephalon > cerebral cortex = olfactory bulb > olfactory tubercle (Monsma et al., 1993). Ruat et al. (1993a) also reported

a very similar distribution, noting highest expression in dorsal and ventral striatum, olfactory tubercle, and hippocampus, with lower expression in stomach and very low to no expression detected in a variety of other peripheral tissues. Radioligand binding and immunohistochemical localization supported a similar distribution in rat and human brain (Gerard et al., 1997; Marazziti et al., 2013b). Using RT-PCR, Hirst et al. (2003) detected a similar distribution of 5-HT_6 receptor mRNA in various regions of rat and human brain, whereas mouse brain had much lower apparent levels of 5-HT_6 receptor mRNA and, unlike in other species, no enrichment in the basal ganglia. Specific binding of [^{125}I]-SB258585, a highly selective 5-HT_6 receptor radioligand, displayed the same patterns of regional localization, with substantially higher levels of 5-HT_6 receptors in striatum than in other regions in rats and humans but fairly homogenous levels of expression in all of the mouse brain regions that expressed 5-HT_6 receptors. Differences in 5-HT_6 receptor pharmacology between these species were evident but did not explain the disparate distributions of receptor expression, highlighting important species differences in 5-HT_6 receptor distribution (and pharmacology).

The cellular profile of expression has been examined in some detail in several brain regions. In the hippocampus and cortex, 5-HT_6 receptor mRNA is present in glutamatergic neurons containing vGluT1 mRNA as well as a subset of GABAergic interneurons that coexpress the 5-HT_3A receptor; 5-HT_6 receptor mRNA is also detected in other classes of interneurons at a lower frequency (Helboe et al., 2015). 5-HT_6 receptor immunoreactive glial cells have also been reported in human cortex (Lorke et al., 2006; Marazziti et al., 2013b). In striatum, 5-HT_6 receptor mRNA is expressed in both direct and indirect pathway medium spiny neurons (Ward and Dorsa, 1996; Helboe et al., 2015) and occasionally in cholinergic interneurons (Bonsi et al., 2007; Helboe et al., 2015).

5-HT_6 receptors are not presynaptic autoreceptors on 5-HT neurons, as 5-HT_6 receptor mRNA in rat is not altered by ablation of the DRN with 5,7-dihydroxtryptamine (Gérard et al., 1996) and is not colocalized with SERT mRNA (Helboe et al., 2015). Nonetheless, their abundant expression on forebrain cortical neurons thought to project to DRN 5-HT neurons may explain how 5-HT_6 receptor ligands can modify 5-HT neuronal firing (see *Electrophysiology* below).

The localization of 5-HT_6 receptors within neurons is still somewhat controversial, perhaps because of differences in antibody specificity. Initial studies described

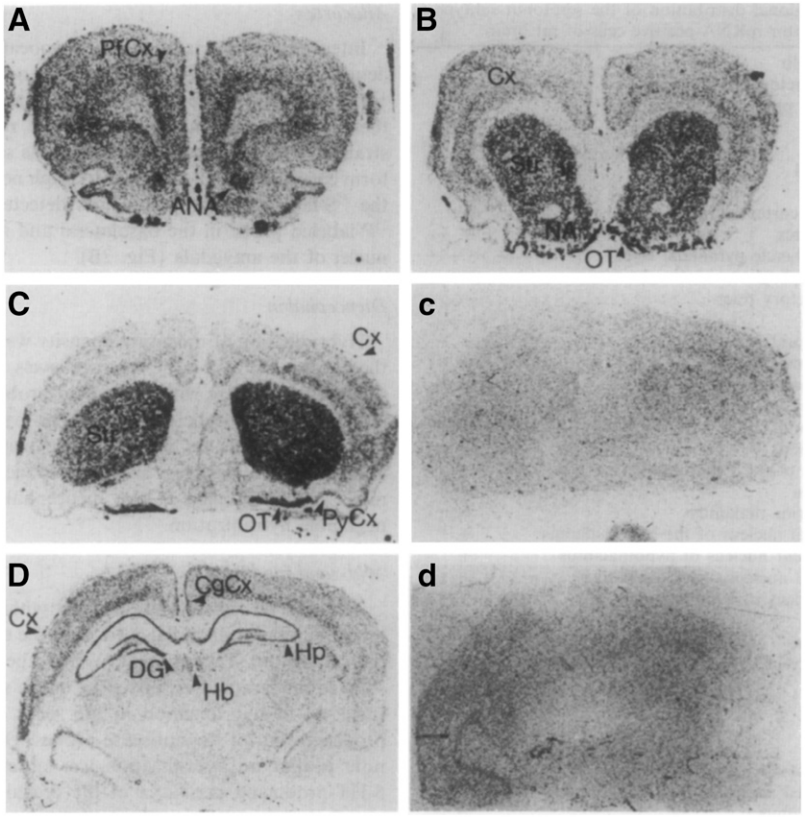

Fig. 25. In situ hybridization detection of 5-HT_6 receptor mRNA expression in rat brain. Autoradiographic visualization of 5-HT_6 receptor mRNA in rat brain (A–D) and relative absence of signal when adjacent sections probed with the sense control (c and d). ANA, anterior nucleus accumbens; CgCx, cingulate cortex; Cx, cortex; DG, dentate gyrus; Hb, habenula; Hp, hippocampus; OT, olfactory tubercle; PfCx, prefrontal cortex; PyCx, pyriform cortex. Adapted from Ward et al. (1995) (with permission).

5-HT_6 receptors in the neuropil and electron microscopy suggested that immunolabeling was predominantly associated with dendrites (Gerard et al., 1997). A subsequent electron microscopy study revealed that 5-HT_6 receptor immunolabeling is not only associated with dendrites but also with primary neuronal cilia (Hamon et al., 1999; Brailov et al., 2000). This is a surprising result because there are few GPCRs that localize to primary neuronal cilia (e.g., somatostatin sst3 receptor), and the 5-HT_6 receptor is the only 5-HT receptor that may do so (Berbari et al., 2008). The ciliary localization may require the presence of key amino acids in the third intracellular loop of the receptor, which is the case for 5-HT_6 receptors in mice, rats, and humans (Berbari et al., 2008). This subcellular distribution may have implications for interactions with other signaling proteins, such as adenylyl cyclase 3, which is localized exclusively in primary cilia (Baker et al., 1998), and resultant functional consequences of 5-HT_6 receptor signaling. However, the distribution and function of 5-HT_6 receptors that are heterologously expressed may be strongly dependent on a number of factors, including the differentiation state of the neuron and the amount of receptor expressed. As an example, exogenous overexpression of transfected 5-HT_6 plasmid DNA has a strong effect on cilia localization, with heavy expression leading to increased rates of nonciliary localization.

C. Pharmacology

There are important species differences between mammalian 5-HT_6 receptors. The mouse 5-HT_6 receptor has similar sequence homology to the homologous receptor of human, pig, or rat, but the pharmacological profile is much closer between human and rat than mouse (Setola and Roth, 2003). Hirst et al. (2003) detected marked differences in the affinity of a number of ligands for the 5-HT_6 receptor in these three species. For example, the antagonist compound Ro 04-6790 binds to recombinant rat or human 5-HT_6 receptors with similar affinity (pK_i ~7.4) but has relatively very low affinity for mouse 5-HT_6 receptor (pK_i < 4); several other antagonists (including SB-258585 and mianserin) have lower affinities (5- and 12-fold, respectively) at mouse compared with rat or human receptors (Hirst et al., 2003); these differences are explained to some degree by variances in the binding pocket as demonstrated by site-directed mutagenesis. On the other hand, several highly selective agonists have similar affinities for mouse, human, and rat 5-HT_6 receptors.

There are relatively far lower levels of 5-HT_6 receptor radioligand binding sites in mouse brain, with lesser density variation across regions, than is evident in rat and human brain. Importantly, this reduced level of receptor binding and rather homogenous density across the mouse brain is method-independent, as it is equally observed using a variety of techniques to localize the receptors. Differences in residues 188 in TM5 and 290 in TM6 contribute to these species differences.

A null mutation (knockout) for the mouse 5-HT_6 receptor results in only subtle changes to the physiologic and behavioral phenotype (Bonasera et al., 2006), consistent with a limited contribution of the 5-HT_6 receptor to baseline mouse behavior. Thus, rats may be a more useful model for studying the pharmacological and (patho)physiogical roles of 5-HT_6 receptors in relation to humans, whereas the availability of a knockout strain makes mice very useful for studying other aspects of the biology of this receptor.

Several strategies for radiolabeling 5-HT_6 receptor have been described. [^{125}I]-SB258585 (Hirst et al., 2006) is the most selective, commercially available option; other radioligands require masking of non–5-HT_6 receptor sites and include [^{3}H]-LSD (Sleight et al., 1998), [^{3}H]-clozapine (Glatt et al., 1995), and [^{11}C]-GSK215083 (Parker et al., 2012, 2015).

Several selective 5-HT_6 receptor agonists are available. 2-Ethyl-5-methoxy-N,N-dimethyltryptamine was the first moderately selective agonist developed (Glennon et al., 2000); it is brain penetrant and has >10-fold selectivity for 5-HT_6 compared with other 5-HT receptors. More selective agonists include WAY181187, WAY208466 (Schechter et al., 2008), and ST1936 (Borsini et al., 2015). Some of these ligands display partial agonism under certain circumstances, such as EMD386088 (Jastrzebska-Wiesek et al., 2013) and E6801 (Romero et al., 2006); many nonselective 5-HT receptor agonists are either full or partial agonists at 5-HT_6 receptors, which can be useful tools in specific cases, but care must be given to rule out actions at other 5-HT receptors. For instance, EMD386088 has moderate affinity (IC_{50} = 34 nM) for the 5-HT_3 receptor (Mattson et al., 2005), and ST1936 has moderate affinity for 5-HT_7, 5-HT_{2B}, and α2-adrenoceptors (K_i = 168, 245, and 300 nM, respectively; Riccioni et al., 2011). However, the contribution of partial agonism to the behavioral or physiologic effects of 5-HT_6 receptor ligands has not been thoroughly characterized.

A number of clinically effective drugs have affinity for 5-HT_6 receptors but also for other receptor targets. Several antidepressants and antipsychotics have high affinity for 5-HT_6 receptors (Monsma et al., 1993; Ruat et al., 1993a), but none of these are selective for the receptors. After the discovery of the 5-HT_6 receptor, a number of modestly selective antagonists were reported based on high-throughput screening of chemical libraries, although these often shared affinity for other 5-HT or dopamine receptors (Upton et al., 2008). The first CNS penetrant selective 5-HT_6 receptor antagonists were produced by Roche (e.g., Ro 04-6790; Sleight et al., 1998) and GlaxoSmithKline (e.g., SB-271046; Bromidge et al., 1999). Subsequently, more highly selective 5-HT_6 receptor antagonists were developed, which are useful for both in vitro and in vivo

experimentation. Among these are SB258585, SB399885 (Hirst et al., 2003), and Ro4368554 (Lieben et al., 2005).

In heterologous systems expressing wild-type or constitutively active 5-HT_6 receptors, a number of the selective agonists may show partial agonist activity, whereas numerous antagonists display inverse agonist properties (Purohit et al., 2003; Romero et al., 2006).

The first 5-HT_6 receptor somewhat selective PET ligand, [^{11}C]-GSK215083 (with approximately fivefold-lower affinity for the $5HT_{2A}$ receptor), demonstrates 5-HT_6 receptor occupancy in the human striatum (Parker et al., 2012, 2015) and will no doubt help to investigate the role of this receptor in human pathology and in therapeutic response/target engagement of existing or new therapeutics or drugs.

D. Post-translational Modifications and Protein Interactions

There is a predicted glycosylation site in the amino terminal of the 5-HT_6 receptor and a number of predicted phosphorylation sites (Monsma et al., 1993; Ruat et al., 1993a; Kohen et al., 1996, 2001), although none of these have been examined systematically. However, phosphorylation of serine 350 appears to be mediated by cyclin-dependent kinase 5 (Cdk5); treatment of NG108 cells with a 5-HT_6 receptor–selective agonist leads to coimmunoprecipitation of 5-HT_6 receptor and Cdk5, whereas the 5-HT_6 receptor–selective antagonist SB258585 reduces the receptor phosphorylation and coimmunoprecipitation with Cdk5 (Duhr et al., 2014). Furthermore, bioluminescence energy transfer experiments confirm the direct interaction of this receptor and Cdk5 in transfected NG108 cells, which is also inhibited by the 5-HT_6 receptor antagonist SB258585. Duhr et al. (2014) demonstrated that Cdk5-dependent phosphorylation of the 5-HT_6 receptor leads to activation of Cdc42 (a Rho GTPase), which can in turn activate further downstream mechanisms. The role of serine 350 phosphorylation was confirmed by site-directed mutagenesis, which may result in constitutive activity of the receptor in the absence of agonist and inhibition of phosphorylation and downstream events by the 5-HT_6 receptor–selective antagonist SB258585. These signaling events may enhance NG108 cell differentiation and neurite outgrowth both in this cell line and in striatal and cultured hippocampal and striatal neurons.

Using HEK293 cells transfected with epitope-tagged 5-HT_6 receptors, Marin's group also detected direct interactions of this receptor with mTOR and several related proteins in the mTOR complex 1 (Meffre et al., 2012). 5-HT_6 receptor–selective agonists induce mTOR activation, social cognition impairments in mice, and object recognition impairment in rats that could be blocked with the mTOR antagonist rapamycin. In summary, these data suggest that direct protein-protein interactions involving the 5-HT_6 receptor may lead to activation of multiple signaling cascades in addition to the canonical G_s-mediated activation of adenylyl cyclase (see *XVII. C. 5-HT_6 Receptor Receptosome: Toward New Signaling Mechanisms Underlying Its Control of Cognition and Neurodevelopmental Processes* for further discussion of protein-protein interactions with the 5-HT_6 receptor).

Though not examined in detail, dimerization of 5-HT_6 receptors can be inferred from the immunoprecipitation and Western blot analysis (Meffre et al., 2012; Duhr et al., 2014).

E. Signaling Pathways

There is growing appreciation for the complexity of the pharmacological and signaling properties of the 5-HT_6 receptor. The ability of the 5-HT_6 receptor to activate adenylyl cyclase in a G_s-dependent manner was first described when the receptor was originally cloned and expressed heterologously as well as in native tissues (Monsma et al., 1993; Ruat et al., 1993a; Sebben et al., 1994; Unsworth and Molinoff, 1994; Choi et al., 2007; Kim et al., 2014b). 5-HT_6 receptors can activate several adenylyl cyclase isoforms, including AC3, AC5, and AC8, but not AC1 or AC8 (Baker et al., 1998). Because 5-HT_6 receptors activate adenylyl cyclase, thereby increasing cAMP and therefore protein kinase A activity, a number of additional downstream signaling consequences can be presumed, although these have not been systematically examined. Svenningsson et al. (2002) examined the potential synergistic interactions between 5-HT_6 and D_1 dopamine receptors; 5-HT_6 receptor activation in striatum regulates $DARPP_{32}$, an enzyme previously associated mainly with dopaminergic neurotransmission. Selective 5-HT_6 receptor agonists modulate the $DARPP_{32}$ phosphorylation state in a manner consistent with increasing this enzyme's activity; phosphorylation of $DARPP_{32}$ is blocked by selective 5-HT_6 receptor antagonists; 5-HT–mediated c-Fos activation and motor activity is blunted in $DARPP_{32}$ knockout mice (Svenningsson et al., 2002). However, 5-HT regulation of $DARPP_{32}$ is unlikely to involve exclusively 5-HT_6 receptors, as 5-HT_4 and 5-HT_7 receptors can also activate $DARPP_{32}$. This pathway is an interesting regulatory node that potentially integrates 5-HT and dopamine signaling in neurons that coexpress 5-HT_6 and D_1 receptors, such as striatonigral (direct pathway) medium spiny neurons.

The 5-HT_6 receptor induces fyn kinase activation. (Yun et al., 2007; Riccioni et al., 2011). Fyn is an src tyrosine kinase family member, present in both cilia and neuronal soma. Fyn is expressed in the same brain regions as 5-HT_6 receptor. Phosphorylated-Fyn is thought to activate Erk1/Erk2 kinases via the Ras-Raf-MEK pathway. Direct protein interactions with Cdk5 also predict that 5-HT_6 receptors signal through this kinase pathway, which was shown to regulate neurite outgrowth in NG108 cells and primary neurons (Duhr et al., 2014).

This pathway regulates neuronal migration in both slice cultures and in vivo (Jacobshagen et al., 2014). 5-HT_6 receptor activation phosphorylates doublecortin and focal adhesion kinase, two mediators of neuronal migration, and is Cdk5-dependent, although additional or alternative mediators may be at play. Similarly, mTOR interactions were predicted based on studies of protein interactions, and 5-HT_6 receptor agonists induce phosphorylation of mTOR into the active form, which is blocked by selective 5-HT_6 receptor antagonists both in vitro and in vivo (Meffre et al., 2012).

The constitutive activity of 5-HT_6 receptors represents an intriguing issue. Constitutive activity implies that the receptor couples to and activates downstream effectors in the absence of the agonist; the clearest evidence for this with 5-HT_6 receptors comes from heterologous expression systems and with activation of Cdk5, Fyn, and mTOR signaling events (Meffre et al., 2012; Duhr et al., 2014; Jacobshagen et al., 2014; see *XVII. C. 5-HT_6 Receptor Receptosome: Toward New Signaling Mechanisms Underlying Its Control of Cognition and Neurodevelopmental Processes*). However, it has been more difficult to confirm this in systems that are dependent on activation of 5-HT_6 receptors expressed in native cells or tissues. For example, Sebben et al. (1994) described 5-HT_6 receptor–mediated cAMP accumulation in primary cultured mouse striatal neurons, and several full agonists, such as 5-HT and LSD, caused cAMP production, but no constitutive activity was apparent. Because the cell type, signal transduction pathways, and level of receptor expression may all affect the extent of constitutive activity, its contribution to 5-HT_6 function likely varies a great deal depending on the cellular context; hence, it still remains to be demonstrated with natural expression levels in vivo. However, the 5-HT_6 receptor clearly has a propensity to display constitutive activity, and many of the available antagonists are in fact inverse agonists, meaning they will reduce 5-HT_6 signaling if constitutive activity is present (Purohit et al., 2003).

F. Function

Prior to the availability of selective 5-HT_6 receptor antagonists, antisense oligonucleotides were used to reduce expression/function of the 5-HT_6 receptor; yawning and stretching behavior (Bourson et al., 1995), weight loss, and reduced retention of spatial learning in the Morris water maze were reported following receptor knockdown (Woolley et al., 2001), later replicated by the use of selective antagonists. Interestingly, the extent of 5-HT_6 receptor knockdown was limited (20%–30% using [^{3}H]LSD binding), and it is only more recently that knockout mice have been available. Tecott and Brennan (2000) produced the first 5-HT_6 receptor knockout mouse and reported weight loss in a patent, which triggered the exploration of 5-HT_6 receptor antagonists as appetite suppressants (Heal et al., 2008; Higgs et al., 2016). More recently, germ-line knockout of 5-HT_6 receptors produced no changes in viability, health, weight, or longevity of the null mutant mice (Bonasera et al., 2006) and no deficits in fertility or maternal behavior. Furthermore, no overt changes in circadian activity, emotional behavior, sensorimotor gating, or cognition were detected. The only behavioral change evident was a reduced sensitivity to the sedative and ataxic effects of acute ethanol administration. However, mouse 5-HT_6 receptor KO may underestimate the potential roles of 5-HT_6 receptors in any of these effects, as mice have much lower 5-HT_6 receptor expression than many other mammalian species (Hirst et al., 2003), as discussed above.

Though 5-HT_6 null mutant mice seem to develop normally, there are very interesting effects of acute modulation of 5-HT_6 receptors during cortical development. Using both in vivo and explant culture models, Dayer and colleagues have shown that modulating 5-HT_6 receptor signaling produces deficits in neuronal migration (Jacobshagen et al., 2014). The 5-HT_6 receptor partial agonist EMD386088 delays the rate of pyramidal neuron and interneuron migration (Riccio et al., 2009, 2001), which can be blocked by the antagonist SB 258585, although the concentrations of drugs used are rather high, questioning receptor classification.

The effect of activation versus inhibition of 5-HT_6 receptor activity is complex, though, as short hairpin RNA knockdown of endogenous 5-HT_6 receptors produces deficits in neuronal speed and extent of neuronal migration (Jacobshagen et al., 2014). This deficit is partially rescued by coexpressing 5-HT_6 receptors or overexpressing Cdk5 but not by coexpressing 5-HT_6 receptor mutants that disrupt Gs-coupling and thus adenylyl cyclase activation. Furthermore, 5-HT_6 receptor–mediated Cdk5 signaling facilitates dendritic outgrowth. There may be additional mechanisms of 5-HT_6 receptor–mediated developmental effects, as the selective agonist WAY181187 activates mTOR in mouse cortex, and this is blocked by SB258585 (Meffre et al., 2012). The psychomimetic drug phencyclidine, which models aspects of the development and symptoms of schizophrenia, induces mTOR activation in neonatal rat cortex and impairs social cognition in the adult mice, both of which are blocked by the mTOR inhibitor rapamycin or the 5-HT_6 receptor–selective antagonist SB258585. Interestingly, both drugs also reverse visual recognition impairments associated with isolation rearing in rats (Marsden et al., 2011; Meffre et al., 2012). Though adenylyl cyclase, mTOR, Cdk5, and fyn kinase signaling are complex and impacted by many parallel pathways, the potential that multiple signaling pathways emanating from 5-HT_6 receptors play a crucial role in establishing normal cortical circuitry has far-reaching implications given that drugs that modulate 5-HT dynamics (such as SSRIs and other antidepressants) or that directly interact with 5-HT_6 receptors (whether exclusively so or not) may

produce alterations in cortical development. These issues need to be examined more fully to understand the consequences of 5-HT drug treatments during gestation or in adolescence.

It is reasonable to conclude that 5-HT_6 receptors may increase the excitability of neurons, as this is the expected result following activation of adenylyl cyclase. The 5-HT_6 receptor agonist WAY181187 increases fos-like immunoreactivity in cortex (Burnham et al., 2010), and the 5-HT_6 receptor antagonist Ro4368554 reduces scopolamine-induced increase of fos-like immunoreactivity in lateral amygdala (Mitchell et al., 2009b); however, both of these manipulations produce complex behavioral and pharmacological changes that make it difficult to conclude that the effects are directly related to 5-HT_6 receptors or are more indirect consequences of circuitry level events.

5-HT_6 receptors modulate many different neurotransmitter systems (Mitchell and Neumaier, 2005; Dawson, 2011). The impacts on extracellular levels of acetylcholine, glutamate, GABA, dopamine, and norepinephrine have been reported using microdialysis. Selective 5-HT_6 receptor ligands alter neuronal firing in a variety of brain regions, but it is difficult to conclude whether these effects are direct or indirect. In any event, it appears that 5-HT_6 receptor ligands modulate numerous brain regions and neurotransmitter systems.

There is a rich literature addressing the potential cognitive effects of 5-HT_6 receptor modulation (Woolley et al., 2004; Mitchell and Neumaier, 2005; King et al., 2008; Codony et al., 2011; Marsden et al., 2011; Meneses, 2015). In most cases, 5-HT_6 receptor antagonists display procognitive effects in a variety of rodent memory tasks, such as spatial learning, novel object recognition, social discrimination, and autoshaping (Mitchell and Neumaier, 2005). Generally, these experiments used parenteral drug administration either just before or just after a memory "training" session that was then assessed after a short interval. The procognitive effects of 5-HT_6 receptor antagonists have often been shown to be associated with changes in other neurotransmitter systems, especially acetylcholine, glutamate, or GABA release. However, it is difficult to reach mechanistic conclusions based on the correlative nature of these observations. At this time, it is not possible to attribute the memory-enhancing effects of 5-HT_6 receptor antagonists to a single brain region or neurotransmitter system, and it seems likely that multiple mechanisms are responsible.

Although there are numerous reports that inhibiting 5-HT_6 receptors promotes (or restores) memory, there is some apparent inconsistency in the cognitive role of 5-HT_6 receptor agonists. For example, WAY117187 (an agonist) impaired social recognition in an antagonist-sensitive manner whether given systemically or locally into frontal cortex (Loiseau et al., 2008); EMD386088 or WAY466 impaired memory in the autoshaping or social recognition tasks, respectively (Meneses et al., 2008; Schechter et al., 2008). However, other reports have observed procognitive effects of 5-HT_6 receptor agonists (Kendall et al., 2011; Woods et al., 2012; Pereira et al., 2015). There are several potential explanations for these discrepancies. One explanation is that 5-HT_6 receptor activation in some brain regions is procognitive, whereas inhibition of 5-HT_6 receptors in other brain regions is also procognitive. Another possible explanation is that the effect of increased or decreased 5-HT_6 receptor activity is entirely dependent on the specific cognitive domain being tested. A third possibility is that either high or low levels of 5-HT_6 receptor activity enhance cognition in specific regions, albeit by different neurochemical mechanisms (e.g., facilitating glutamatergic function in states with a relative deficit or facilitating GABAergic function in states of excessive glutamatergic activity). Even when the same cognitive task has been used, each laboratory likely performs these tests in at least slightly different ways —with or without amnestic maneuvers (such as using scopolamine to disrupt memory function), in young or old animals, or with short- versus long-interval testing; thus, it seems unlikely that agonists or antagonists will "win" this battle, but instead there may be a role for both 5-HT_6 receptor agonists and antagonists for different cognitive problems in different pathologic states.

The effects of striatal 5-HT_6 receptors on autoshaping and related instrumental learning tasks have been investigated based on the early observations that 5-HT_6 receptor antagonists facilitate cholinergic function (Mitchell and Neumaier, 2005); it was thought that these drugs facilitate consolidation of memory by cortical or hippocampal mechanisms. However, because 5-HT_6 receptors are most heavily expressed in medium spiny neurons in striatum, these neurons might also be an important site of 5-HT_6 receptor action in cognition. Because the autoshaping task used extensively by Meneses' group was clearly sensitive to 5-HT_6 receptor antagonism, this task was studied in mice with striatum-specific increased 5-HT_6 receptor expression (Meneses 2015; Mitchell et al., 2007). High 5-HT_6 receptor density in dorsomedial striatum impaired the acquisition but not the expression of previously learned instrumental learning. This effect is unlikely to involve memory consolidation, as the deficit is only reversed by giving a 5-HT_6 receptor antagonist before but not after the training session. The operant conditioning parameters were altered to allow single-session acquisition; increased 5-HT_6 receptor expression interfered with learning under these conditions (Eskenazi and Neumaier, 2011b). Furthermore, the type of learning affected by increasing the local expression of 5-HT_6 receptors depended entirely on the subregion of striatum targeted (Mitchell et al., 2007; Ferguson et al., 2008; Eskenazi and Neumaier, 2011a,b). These studies do not exclude a role of 5-HT_6 receptors in cholinergic interneurons in striatum,

as the focus was on the direct and indirect striatal output pathways, which have generally opposing effects on behavior (Yager et al., 2015). Increased 5-HT_6 receptor expression in indirect pathway medium spiny neurons is sufficient to disrupt instrumental learning in dorsomedial striatum or to facilitate learning of new behavior in overtrained animals when the dorsolateral striatum was targeted (Eskenazi et al., 2015). Indeed, 5-HT_6 receptors tend to oppose the effects of dopamine on striatal-based behaviors including learning, as they are expressed in both output pathways, whereas D_1 and D_2 dopamine receptors are differentially expressed in these pathways (Gerfen and Surmeier, 2011). These subtleties illustrate how 5-HT_6 receptors can have diverse effects on behavior depending on the cells that express these receptors. Thus, a full understanding of how 5-HT_6 receptors modulate learning and memory in a specific cognitive disorder may depend, at least in part, on how that disorder changes 5-HT receptor signaling.

In addition to effects on cognition, 5-HT_6 receptors have been proposed to promote satiety and reduce feeding and body weight (Woolley et al., 2001; Fisas et al., 2006; Voigt and Fink, 2015; Higgs et al., 2016); however, not all studies report effects of chronic 5-HT_6 receptor antagonism on body weight in rats or humans (Mitchell et al., 2009; Wilkinson et al., 2014; Quiedeville et al., 2015). Most of these studies used animals with normal body weight, whereas the 5-HT_6 receptor antagonist idalopirdine reduced feeding, peritoneal fat, and body weight when rats were fed a high-fat diet (Dudek et al., 2015). This metabolic profile might be advantageous clinically, as reducing feeding only in obese individuals would mitigate the risks of the drug when used for treating individuals with dementia. Furthermore, as several atypical antipsychotics that have high affinity for 5-HT_6 receptors (along with many other sites) are associated with increased appetite, weight gain, and the development of the metabolic syndrome, the preclinical and limited clinical data available suggest that 5-HT_6 receptor antagonism is unlikely to be responsible for these adverse effects, which are more likely due, at least in part, to 5-HT_{2C} receptor antagonism.

The 5-HT_6 receptor has also been examined in relation to drug dependence given their dense expression in the striatum, a key mediator of reward seeking and habit formation. However, some reports have found no effect of systemic 5-HT_6 receptor ligands on cocaine self-administration (Frantz et al., 2002; Fijał et al., 2010; Valentini et al., 2013), whereas others suggest that cocaine reinforcement or reinstatement is regulated by 5-HT_6 receptor activity (van Gaalen et al., 2010; Valentini et al., 2013). Increased striatal 5-HT_6 receptors modulate learning of drug-associated behaviors as well as the stability of habitual responding (Ferguson et al., 2008; Eskenazi and Neumaier, 2011a; Eskenazi et al., 2015), but selectively increasing 5-HT_6 receptors in the indirect pathway medium spiny neurons in ventral striatum increases the sensitivity to the reinforcing properties of self-administered cocaine (Brodsky et al., 2016).

5-HT_6 receptors may also play a role in the development and treatment of epilepsy (Hirst et al., 2006). Increased 5-HT_6 receptor levels were detected in brain tissue surgically removed from humans with epilepsy and in the pilocarpine model of epilepsy in rats (Wang et al., 2015). The selective antagonist SB399885 reduces mTOR activation and epileptic activity in this animal model. Furthermore, the colocalization of 5-HT_3A and 5-HT_6 receptors in a subset of cortical interneurons (Helboe et al., 2015) is intriguing given the potential role of these interneurons in cortical excitability for both normal cognition as well as pathologic states.

1. Electrophysiology. Much of the electrophysiological data concerning 5-HT_6 receptors primarily concerns in vivo recording; the cellular mechanisms are difficult to infer. The selective 5-HT_6 receptor agonist WAY181187 increases excitability and spiking by rat medium spiny neurons in acutely prepared striatal slices in a SB258585-reversible manner; but this has not been sufficiently explored to identify whether this is a direct or indirect phenomenon. In whole-cell patch clamp mode, the 5-HT_6 receptor agonist ST1936 reduces spontaneous excitatory postsynaptic currents in striatal medium spiny neurons and cortical pyramidal neurons, which is prevented by SB258585 (Tassone et al., 2011). Thus, the agonist-induced reduction in glutamatergic neuronal activity (consistent with microdialysis reports of enhanced glutamate overflow following microinfusion of 5-HT_6 receptor antagonists; Upton et al., 2008; Dawson, 2011) might be indirect by modulation of GABAergic interneurons in these brain areas. Although systemic administration of 5-HT_6 receptor agonists to anesthetized rats produced both excitation and inhibition of ventral tegmental area (VTA) dopamine neurons, only excitation is observed following microiontophoretic application (Borsini et al., 2015), suggesting a direct excitatory activation, which may be relevant for cognitive and addictive properties of 5-HT_6 receptor ligands.

Moderate levels of 5-HT_6 receptors are also expressed in the DRN, and local injection of the agonist WAY-208466 decreases REM sleep and increases wakefulness (Monti et al., 2013). Single-unit recording in the anesthetized rat shows that systemic application of the full agonist WAY-181187 increases and the antagonist SB-399885 decreases firing of putative DRN 5-HT neurons; further studies are needed to establish if this involves cortical feedback loops, interneurons, and/or a direct effect on 5-HT neurons as proposed by the authors (Brouard et al., 2015). Consistent with this idea of indirect modulation of 5-HT release in the raphe, the 5-HT_6 receptor antagonist SB399885 inhibits spontaneous firing of GABAergic interneurons (expressing 5-HT_6 receptor mRNA) in the DRN in mouse brain slices (Asaoka et al., 2015). This mechanism may be

relevant to the action of antipsychotic drugs, as the effect is mirrored by olanzapine.

G. Clinical Relevance

Treatment of cognitive impairment is a leading potential application of 5-HT_6 receptor ligands in humans. There is ample evidence that manipulation of 5-HT_6 receptors can improve cognitive function based on preclinical animal models. Though schizophrenia is associated with positive and negative symptoms that can respond to available antipsychotic drugs, cognitive impairment is an often disabling and persistent problem that typically does not respond well to antipsychotic medications. Although there are some preliminary clinical trials in schizophrenia, these trials had technical limitations because the 5-HT_6 receptor ligands investigated were as add-ons to treatments already underway, and in some cases, the ongoing treatments were atypical antipsychotic drugs that possess potent antagonist properties at 5-HT_6 receptors, making it unlikely that additional benefits from 5-HT_6 receptor blockade could be readily detected. Early clinical studies with 5-HT_6 receptor ligands are reviewed elsewhere (Heal et al., 2008; Codony et al., 2011). A double-blind, placebo-controlled phase II trial for treatment of dementia associated with Alzheimer Disease (Wilkinson et al., 2014) investigated whether the addition of Lundbeck's idalopirdine, a selective 5-HT_6 receptor antagonist, to an established treatment (donepezil, a cholinesterase inhibitor) delays the progression of dementia. This study was successful in two important ways: the addition of idalopirdine was well tolerated and led to improved cognitive function as compared with addition of placebo. However, disappointingly, idalopirdine suffered a late-stage failure in a pivotal phase III trial. Along the same lines, intepirdine, previously known as RVT-101, a 5-HT_6 receptor antagonist originally developed by GSK, displayed positive effects in patients with AD in a phase II clinical trial. A subsequent phase III trial of the drug after it was acquired by Axovant failed to achieve any of its main efficacy targets in mild to moderate AD patients. Intepirdine did not improve symptoms compared with placebo on two widely used AD symptom measures—the ADAS-Cog (the Alzheimer's Disease Assessment Scale–Cognitive Subscale) and ADCSADL (Alzheimer's Disease Cooperative Study Activities of Daily Living Scale) scales. Subsequently, intepirdine was evaluated in a clinical trial of patients with dementia with Lewy bodies also without success, leading to a halt in the development of this drug. Hence, although the availability of new effective treatments for dementia is a crucial health imperative, it would appear that 5-HT_6 receptor antagonism may not transform the preclinical promises into clinical practice; understanding the mechanisms that underlie this apparent failure is important and may allow opportunity for patient stratification to better identify patients that may respond favorably to 5-HT_6 receptor ligands.

Several other pathologies might also be amenable to treatment with 5-HT_6 receptor ligands, as preclinical data suggests that other progressive dementing disorders, depression, obesity, and epilepsy have potential to become indications for 5-HT_6 receptor ligands. As now appreciated, the multiple signal transduction pathways engaged by 5-HT_6 receptors and various 5-HT_6 receptor ligands with differing degrees of intrinsic efficacy; the role of agonism partial agonism, antagonism, and inverse agonism; and biased signaling need to be considered in future development of 5-HT_6 receptor therapeutic agents. Such pharmacological complexity may also account for the apparent opposing actions of 5-HT_6 receptor ligands in preclinical behavioral models as discussed above.

XV. 5-HT_7 Receptors

A. Introduction

The 5-HT_7 receptor was the last 5-HT receptor to be discovered. In 1993, several research groups, almost at the same time, identified the 5-HT_7 receptor from the screening of cDNA libraries from various species, including humans. 5-HT_7 receptor mRNA is localized in discrete areas of the mammalian brain, including thalamus, hippocampus, and cortex, and matched with the expression of 5-HT_7 receptor protein. Based on its distribution in the CNS, the 5-HT_7 receptor is proposed to be involved in thermoregulation, circadian rhythm, learning and memory, hippocampal signaling, and sleep. Various drugs (clozapine, cyproheptadine, and amitryptiline) and pharmacological tools (5-CT and 8-OH-DPAT) binding to 5-HT_7 receptor poses the question of whether some of the effects of these compounds are mediated, at least in part, by 5-HT_7 receptor. Initially, the lack of selective 5-HT_7 receptor agonists and antagonists slowed down the elucidation of the (patho)physiologic role of the receptor, especially because of the high sequence homology in the transmembrane domains of 5-HT_7 and 5-HT_{1A} receptors. Moreover, the two receptors are distributed in the same areas of the CNS and, at the cellular level, they may have opposite effects: the 5-HT_7 receptor is positively coupled to adenylyl cyclase whereas the 5-HT_{1A} receptor is negatively coupled. After 2000, various selective antagonists (SB-258719 and SB-269970) and agonists (AS-19, LP-211, and E-55888) became available, providing the scientific community with powerful pharmacological tools to get deeper insights on the role of 5-HT_7 receptors in health and disease.

B. Cloning of the Gene

The 5-HT_7 receptor is a class A GPCR, cloned by screening of cDNA libraries from mouse (Plassat et al., 1993), rat (Lovenberg et al., 1993a, Meyerhof et al., 1993, Ruat et al., 1993b; Shen et al., 1993), human (Bard et al., 1993), guinea pig (Tsou et al., 1994), *Xenopus laevis* (Nelson et al., 1995), pig (Bhalla et al., 2002a),

Caenorhabditis elegans (Hobson et al., 2003), and honeybee (Schlenstedt et al., 2006).

The open reading frame of the human cDNA codes for a protein of 445 amino acids with 57% sequence identity within the transmembrane regions in comparison with the *Drosophila melanogaster* 5-HT_{dro1} receptor and 39%–53% homology with human 5-HT_1, 5-HT_2, 5-HT_5, and 5-HT_6 receptors (Bard et al., 1993). The gene encoding the human 5-HT_7 receptor is located on chromosome 10 (q21-q24) (Gelernter et al., 1995) and contains several introns in the coding region (Ruat et al., 1993b, Erdmann et al., 1996; Heidmann et al., 1997). The guinea pig 5 HT_7 receptor has 466 amino acids, with an amino acid homology within the transmembrane regions with other 5-HT receptors of 34% and 48% (Tsou et al., 1994). The mouse brain 5-HT_7 receptor cDNA has one long open reading frame for 448 amino acids. The homology with other 5-HT receptors is low, with the best score with the 5-HT_{dro1} receptor (42%) and the next closest homology being with 5-HT_{1B}, 5-HT_{1D}, and 5-ht_{1e} receptors (Plassat et al., 1993). The 5-HT_7 receptor was cloned from rat by four groups, which reported different amino acid lengths ranging from 404 to 448. Shen et al. (1993) reported the sequencing of a full-length clone isolated from a rat hippocampal cDNA library, revealing a 404-amino-acid protein with seven hydrophobic regions. Within these regions, rat 5-HT_7 receptor is 44%–50% identical with members of the 5-HT_1, 5-HT_5, and 5-HT_6 subfamilies, with lower homology to the 5-HT_2 receptor subtypes (37%–40%). Lovenberg et al. (1993a) isolated and determined the nucleotide sequence of a clone showing an open reading frame that encoded a 435-amino-acid protein. Within the conserved transmembrane domains of known 5-HT receptors, the rat 5-HT_7 receptor exhibited the greatest identity with the 5-HT_{dro1} receptor (54%). Instead, the entire coding sequence showed low identity (33%–39%) with 5-HT_{dro1}, 5-HT_{1A}, 5-HT_{1D}, 5-ht_{1e}, 5-HT_5 receptor subtypes. Ruat et al. (1993b) reported the characterization of a nucleotide sequence containing an open reading frame encoding a 448-amino-acid protein. This rat 5-HT_7 receptor showed the highest sequence homology in the hydrophobic regions with the 5 HT_{dro1} receptor (60%). In the transmembrane domains, the homologies with other 5-HT receptors were as follows: 5-HT_{1A}, 51%; 5-HT_{1B}, 55%; 5-HT_{1D}, 52%; 5-ht_{1e}, 53%; 5-HT_{1F}, 52%; 5-HT_{2A}, 43%; 5-HT_{2B}, 40%; 5-HT_{2C}, 42%; 5-HT_5, 48%; and 5-HT_6, 45%. The rat 5-HT_7 receptor reported by Meyerhof et al. (1993) has an open reading frame encoding a 448-amino-acid protein, showing the highest sequence homology with 5-HT_{dro1} receptor (36% identity). The 5-HT_7 receptor from pig (Bhalla et al., 2002a) encoded an open reading frame of a 447-amino-acid protein that showed high homology (92%–96%) with the 5-HT_7 receptor protein cloned from the other species.

The presence of introns in the 5-HT_7 receptor gene results in a number of functional splice variants. Although no alternative splicing has been reported for the first intron, located in the sequence encoding the second intracellular loop of the receptor, alternative splicing at the second intron, which is located in the sequence encoding the C-terminal end, generates a number of splice variants, namely 5-$HT_{7(a),\ (b),\ (c),\ (e)}$ receptors in rat and 5-$HT_{7(a),\ (b),\ (d)}$ receptors in man (Heidmann et al., 1998; Krobert et al., 2001; Liu et al., 2001). Splice variants in dog, marmoset, and zebrafish are to be found in the www.ensembl.org database, but no related reports are available in the literature. In the mouse, two additional splice variants have been described, named 5-$HT_{7(b)}$ and 5-$HT_{7(c)}$ in analogy with the rat (Gellynck et al., 2008). In guinea pig and pig, the 5-HT_7 receptor is homologous to the human 5-$HT_{7(a)}$ variant. Although the splice variants differ in the lengths of their carboxy terminal ends, they do not show major differences in their membrane localization nor significant differences in their respective pharmacology and signal transduction properties or functional coupling to G_s protein (see below).

A transcribed human 5-HT_7 receptor pseudogene has been identified by a degenerate PCR approach. The original clone (S771) has homology greater than 90% to the 5-HT_7 receptor sequence; expression of the pseudogene transcript is detected throughout the brain and peripheral tissues, in general agreement with 5-HT_7 receptor mRNA localization. However, the transcript was also detected in tissues not known to express the 5-HT_7 receptor (i.e., liver and kidney) (Olsen et al., 1999).

There is as yet no crystal structure of the 5-HT_7 receptor, but molecular modeling and site-directed mutagenesis has identified essential residues for ligand binding and activation of the human receptor (Impellizzeri et al., 2015).

C. Expression

1. mRNA. The distribution of mRNA encoding the 5-HT_7 receptor protein has been studied in several species using various techniques, as summarized in Tables 18 and 19. In all species, high levels of 5-HT_7 receptor mRNA are expressed in the CNS (hypothalamus, thalamus, and hippocampus; Fig. 26). In peripheral tissues, 5-HT_7 receptor mRNA is present in the ileum, spleen, endocrine glands, and arteries. In blood vessels and the gastrointestinal tract, 5-HT_7 receptor mRNA expression is generally present in smooth muscle cells.

The relative abundance of the three human 5-HT_7 isoforms 5-$HT_{7(a),\ (b),}$ and $_{(d)}$ within brain (fetal brain, caudate, hippocampus) and peripheral tissues (uterus, trachea, small intestine, stomach, saphenous vein) has been examined (Krobert et al., 2001; Guthrie et al., 2005). These tissues expressed all three isoforms. Although the 5-$HT_{7(b)}$ isoform is most prevalent, the relative amounts of 5-$HT_{7(a)}$ and 5-$HT_{7(d)}$ differed by

TABLE 18
Localization and relative abundance of 5-HT_7 receptor mRNA in the rat

Technique	Localization (Relative Abundance)
In situ hybridization[a]	Hippocampus (+++), thalamus (+), hypothalamus (+)
In situ hybridization[b]	Hippocampus, thalamus, enthorinal and piriform cortices, tenia tecta
In situ hybridizatioc[c]	Thalamus (+++), hippocampus (++), retrosplenial cortex (++), neocortex (++), hypothalamus (+) (not detected in suprachiasmatic nucleus)
In situ hybridization[d]	Outer layer of the cortex, thalamus, hippocampus
In situ hybridization[e]	Thalamus (+++), hippocampus (+++), retrosplenial cortex (+++), mammillary region (+++), posterior thalamic nucleus (+), suprachiasmatic nucleus (+)
In situ hybridization[f]	Forebrain (+++), olfactory complex (+++), thalamus (+++), hippocampal formation (+++), hypothalamus (+++), suprachiasmatic nucleus (+++), septal region(++), amygdala (++), parvicellular (++)
In situ hybridization[g]	Retrosplenial cortex (+++), hippocampus (+++), tenia tecta (+++), indusium griseus (+++), posterior hypothalamus (+++), medial amygdala nucleus (+++), thalamus (+++), cerebellum-Purkinije cell layer- (+++), pontine nuclei (+++), superior colliculus (+), raphe nucleus (+)
Northern Blot[h]	Hypothalamus (+++), hippocampus (++), mesencephalon (++), cortex (++), olfactory bulb (+), olfactory tubercle (+), spleen (+) (not detected in retina, pituitary, testis, stomach, prostate, ovary, skeletal muscle, lung, liver, kidney, gut)
Northern Blot[g]	Hypothalamus (+++), brainstem (+++), hippocampus (+++), stomach (+), ileum (+)
Northern Blot[a]	Hypothalamus (+++), thalamus (+++), hippocampus (+), cortex (+) (not detected in urinary bladder, testis, liver, spleen, adrenal gland, kidney, lung, heart, pituitary)
Northern Blot[c]	Hypothalamus (+++), thalamus (+++), hippocampus (++), cortex (++), medulla (++) (not detected in cerebellum, striatum, heart, liver, kidney, adrenal glands, testis, ovaries, spleen)
RT-PCR[i]	Lumbar dorsal root ganglia, superior cervical ganglia, lumbar synpathetic ganglia
RT-PCR[j]	Vena cava (+++), femoral vein (++), aorta (+), renal artery (+), portal vein (+) (not detected in jugular vein)
RT-PCR[k]	Frontocortical astrocytes, hypothalamus
RT-PCR[l]	Adrenal gland
RT-PCR[m]	Submandibular gland
RT-PCR[n]	Thymus, peripheral blood lymphocytes, spleen, mitogen activate spleen cells
RT-PCR[o]	Adrenal cortex
RT-PCR[p]	Jejunum, ileum, stomach fundus, esophagus, colon

[a]Meyerhof et al. (1993).
[b]Kinsey et al. (2001).
[c]Lovenberg et al. (1993).
[d]Mengod et al. (1996).
[e]Venero et al. (1997).
[f]Neumaier et al. (2001).
[g]Ruat et al. (1993b).
[h]Shen et al. (1993).
[i]Pierce et al. (1997).
[j]Ullmer et al. (1995).
[k]Shimizu et al. (1996).
[l]Contesse et al. (1999).
[m]Bourdon et al. (2000).
[n]Stefulj et al. (2000).
[o]Lenglet et al. (2000).
[p]Liu et al. (2001).

tissue type, with the 5-$HT_{7(d)}$ isoform being most abundant in smooth muscle and least common in brain tissues (Krobert et al., 2001; Guthrie et al., 2005). In the rat, the 5-$HT_{7(a)}$ receptor isoform is most prevalent in both the CNS (cerebellum, cortex, hippocampus, hindbrain, thalamus) and the periphery (heart, kidney, spleen) (Heidmann et al., 1997, 1998).

2. Radioligand Binding. Radioligand binding assays have been used to study the distribution of 5-HT_7 receptor protein in native tissues. A first study to define 5-HT_7 receptor binding sites in rat hypothalamic membranes was performed using [^{3}H]5-HT in the presence of 100 nM pindolol that blocks the binding of the radioligand to 5-HT_{1A} and 5-HT_{1B} receptors. The pharmacology of the identified binding sites correlated well with that of rat recombinant 5-HT_7 receptors (Sleight et al., 1995). A subsequent study showed that 100 nM of pindolol does not completely mask 5-HT_{1A} and 5-HT_{1B} receptors and that the population of pindolol-insensitive receptors labeled by [^{3}H]5-HT in rat hypothalamus appeared to be heterogeneous (Gobbi et al., 1996). Also, [^{3}H]5-CT failed to define a homogeneous population of 5-HT_7 binding sites in rat hypothalamus homogenates even in the presence of various masking agents (pindolol, sumatriptan, DOI) (Stowe and Barnes, 1998). [^{3}H]5-CT has been used to label 5-HT_7 receptors in guinea pig brain cortex membranes in the presence of sumatriptan and cyanopindolol (To et al., 1995). The pharmacology of these binding sites was well correlated to that of the guinea pig cloned 5-HT_7 receptor. Because affinity values of reference compounds were consistently lower in the binding assay performed in native tissue, the authors hypothesized that sumatriptan and cyanopindolol were likely to occupy, at least in part, the 5-HT_7 receptors. [^{3}H] Mesulergine labels 5-HT_7 receptors in guinea pig ileal longitudinal muscle in the presence of several masking agents (cinanserin, prazosin, raclopride, RS 102221, and yohimbine). However, under these same conditions, no binding was detected in the rat jejunum (Hemedah et al., 1999).

[^{3}H]SB-269970 is the first selective radioligand to label with high affinity 5-HT_7 receptors in rat, mouse, guinea pig, pig, marmoset, and human brain homogenates. Guinea pig brain homogenate displays markedly higher 5-HT_7 receptor expression in comparison with

TABLE 19
Localization and abundance of 5-HT_7 receptor mRNA in guinea pig, human, mouse, and pig

Species	Technique	Localization (Relative Abundance)
Guinea pig[a]	In situ hybridization	Hippocampus (+++), periventricular thalamus (+++), superficial cortex (+++), cerebellar granule cells (+++)
Guinea pig[b]	In situ hybridization	Medial thalamic nucleus (+++), hippocampal formation (+++), superficial layer cortex (++), medial geniculate nucleus (++), amygdala (++), hypothalamus (++), midbrain (+), hindbrain (+)
Guinea pig[c]	In situ hybridization	Outer layer of the cortex, thalamus, hippocampus
Guinea pig[a]	Northern Blot	Parietal cortex (+++), hippocampus (++), frontal cortex (++), cerebellum (+), ileum (+), spleen (+)
Guinea pig[d]	Northern Blot[b]	Thalamus (+++), brainstem (+++), hypothalamus (+++), substantia nigra (+++), olfactory bulb (+++), olfactory tubercle (+++) (not detected in peripheral organs)
Hamster[e]	In situ hybridization	Thalamus (+++), cortex (++), hypothalamus (++), amygdala (++), midbrain (+), basal forebrain (+)
Human[f]	RT-PCR	Artery smooth cells (+++), pulmonay artery smooth cells (++) (not detected in coronary artery, pulmonary artery, aortic endothelial)
Human[g]	RT-PCR	Dorsal root ganglia
Human[h]	RT-PCR	Granulosa-lutein cells
Human[i]	RT-PCR	Brain (+++), kidney (+), liver (+), pancreas (+), spleen (+), coronary artery (++), stomach (++), descending colon (++), ileum (++)
Human[j]	RT-PCR	Trigeminal ganglia
Human[k]	qPCR	Colonic circular muscle
Mouse[l]	Northern Blot	Not detected in brain, heart, kidney, lung, liver
Mouse[l]	RT-PCR	Forebrain (+++), brain stem (+++), cerebellum (++), colliculi (++), intestine (+), heart (+), not detected in spleen, kidney, lung, liver
Mouse[m]	RT-PCR	Substantia gelatinosa of the trigeminal subnucleus caudalis
Pig[f]	RT-PCR	Pulmonary artery (++), coronary artery (++), cerebral artery (++), cerebral vein (++)
Pig[n]	RT-PCR	Myometrium
Pig[o]	RT-PCR	Brain cortex, trigeminal ganglion, cerebellum, pulmonary artery, coronary artery, superior vena cava, saphenous vein, not detected in heart

[a]Tsou et al. (1994).
[b]To et al. (1995).
[c]Mengod et al. (1996).
[d]Ruat et al. (1993).
[e]Duncan and Franklin (2007).
[f]Ullmer et al. (1995).
[g]Pierce et al. (1997).
[h]Graveleau et al. (2000).
[i]Bard et al. (1993).
[j]Terrón et al. (2001).
[k]Irwing et al. (2007).
[l]Plassat et al. (1993)
[m]Yang et al. (2014).
[n]Kitazawa et al. (2001).
[o]Bhalla et al. (2002).

rat, mouse, and pig brain homogenates (Thomas et al., 2002).

Autoradiographic studies on the localization of 5-HT_7 receptors have been performed by using various radioligands. [^{3}H]5-CT has been used in the presence of various masking agents (To et al., 1995; Waeber and Moskowitz, 1995a; Gustafson et al., 1996; Mengod et al., 1996). In guinea pig and rat brain, the distribution of the 5-HT_7 receptor binding sites was largely consistent with that reported for 5-HT_7 receptor mRNA. The highest densities were in the medial thalamic nuclei and related limbic and cortical regions. However, as the detected densities were very low, it was hypothesized that the masking compounds might have sufficient affinity for 5-HT_7 receptor sufficient to compete with [^{3}H]5-CT to some degree for the 5-HT_7 receptor. In a subsequent study, the localization of 5-HT_7 receptor was studied in 5-HT_{1A} receptor knockout and 5-$HT_{1A/B}$ receptor double-knockout mice by using [^{3}H]5-CT (5-HT_{1A}, 5-HT_{1B}, and 5-HT_7 agonist) and [^{3}H]8-OH-DPAT (5-HT_{1A} and 5-HT_7 agonist) (Bonaventure et al., 2002b). [^{3}H]8-OH-DPAT was better than [^{3}H]5-CT for measuring 5-HT_7 receptor binding sites, even if it displays lower 5-HT_7 receptor affinity than [^{3}H]5-CT in tissue homogenates. The anatomic distribution of the [^{3}H]8-OH-DPAT–labeled sites observed in these knockout mice was in agreement with the distribution of 5-HT_7 receptor mRNA and immunoreactivity reported previously. Within the hippocampal formation, strong labeling was found in the CA3 region, whereas low binding was found in CA1 region. 5-HT_7 receptors were also found within the dorsal raphe and the hypothalamus, including the suprachiasmatic nucleus.

The selective high-affinity radioligand [^{3}H]SB-269970 was used to localize the 5-HT_7 receptors in human brain (Varnäs et al., 2004; Fig. 27). The distribution of the 5-HT_7 receptors was largely similar to that shown by the autoradiographic studies in rat (Gustafson et al., 1996), guinea pig (To et al., 1995), and mouse (Martín-Cora and Pazos, 2004). High receptor density was detected in thalamus, hypothalamus, and hippocampus. However, unlike in rodents, human brain 5-HT_7 receptors were also found in high levels in caudate nucleus, putamen, and substantia nigra (Varnäs et al., 2004). Eventually, using [^{3}H]SB-269970, Horisawa et al. (2013) reported that 5-HT_7 receptor distribution in rat brain was similar

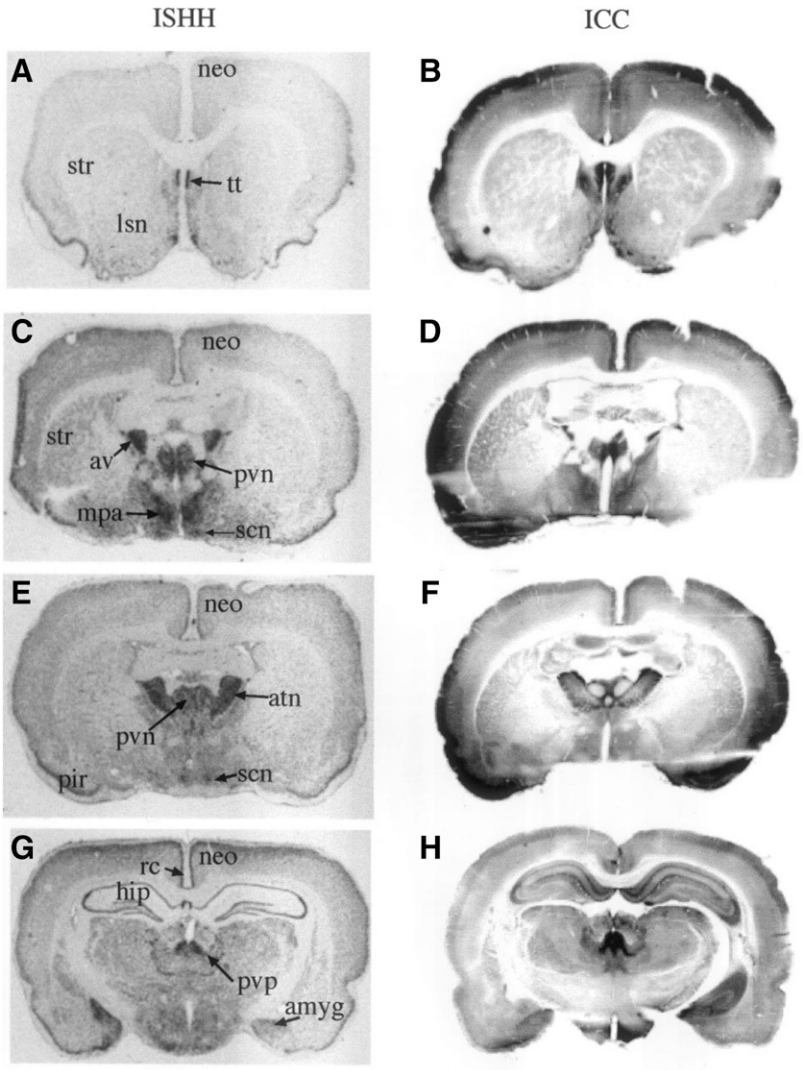

Fig. 26. 5-HT_7 receptor mRNA and protein expression in rat brain. Comparative in situ hybridization localization of 5-HT_7 receptor mRNA (ISHH) (A, C, E, and G) and immunocytochemical location of 5-HT_7 receptor protein (ICC) (B, D, F, and H), respectively. amyg, amygdala; atn, anterior thalamic nuclei; av, anteroventral thalamic nucleus; hip, hippocampus; lsn, lateral septal nuclei; mpa, medial preoptic area; neo, neocortex; pir, piriform cortex; pvp, posterior paraventricular nucleus; pvn, paraventricular thalamic nuclei; rc, retrosplenial cortex; scn, suprachiasmatic nuclei, str, striatum; tt, tenia tecta. Adapted from Neumaier et al. (2001) (with permission).

to that in human (Varnäs et al., 2004), with small differences in regions with an intermediate to low density of 5-HT_7 receptors.

3. Immunoreactivity. Rabbit polyclonal antibodies raised against amino acid sequence 8–23 of the rat 5-HT_7 receptor are commercially available. Immunocytochemistry has been used to localize the distribution of 5-HT_7 receptors in rat forebrain (Neumaier et al., 2001), which were detected in the cortex, hippocampal formation, tenia tecta, thalamus, and hypothalamus, in agreement with the localization reported for the 5-HT_7 receptor mRNA. In particular, in the suprachiasmatic nucleus, both cell bodies and proximal fibers were strongly stained, suggesting a somatodentric subcellular distribution of the receptor. The presence of 5-HT_7 receptors was also reported in both pre- and postsynaptic GABA, vasoactive intestinal polypeptide, and vasopressin processes in the suprachiasmatic nucleus in mouse (Belenky and Pickard, 2001). 5-HT_7 receptors are detected in Purkinje cells of rat cerebellum but not in the cerebellar cortex or in deep nuclei (Geurts et al., 2002).

5-HT_7 receptor immunolabeling was detected mainly in the two superficial laminae of the dorsal horn and in small- and medium-sized dorsal root ganglion cells in the rat spinal cord (Doly et al., 2005), consistent with a predominant role of 5-HT_7 receptor in nociception. In addition, moderate labeling was found in the lumbar dorsolateral nucleus (Onuf's nucleus), suggesting an involvement of the receptor in the control of pelvic floor muscles (Doly et al., 2005). Electron microscopic examination of the dorsal horn shows three main localizations of the receptor: 1) a postsynaptic localization on peptidergic cell bodies in laminae I–III and in numerous dendrites, 2) a presynaptic localization on unmyelinated and thin myelinated peptidergic fibers,

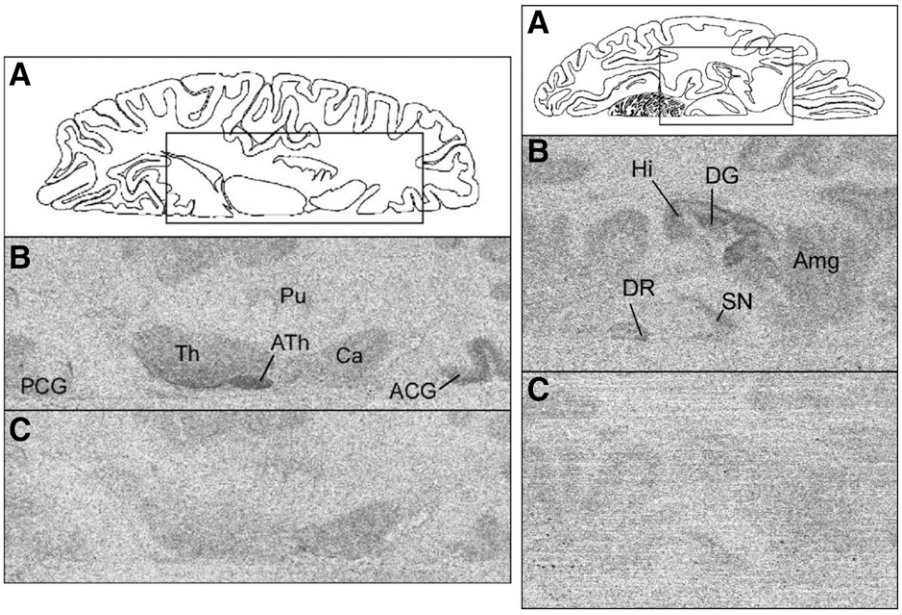

Fig. 27. 5-HT_7 receptor binding sites in human brain. Autoradiographic detection of [^{3}H]SB-269970 binding to 5-HT_7 receptors in the human brain. Autoradiograms showing the distribution of 5-HT_7 receptors at the level of the thalamus (left column) and dorsal striatum (right column). (A) Plates; line drawings of brain hemisphere contours with square showing location of sections for the autoradiograms below. (B) Plates; total binding. (C) Plates; nonspecific binding (inclusion of 5-HT, 10 μM). ACG, anterior cingulate gyrus; Amg, amygdala; Ath, anterior thalamus; Ca, caudate nucleus; DG, dentate gyrus; DR, dorsal raphe; Hi, hippocampus; PCG, posterior cingulate gyrus; Pu, putamen; SN, substantia nigra; Th, thalamus. Adapted from Varnäs et al. (2004) (with permission).

and 3) in lamina I and II in astrocytes (Doly et al., 2005). 5-HT_7 receptors have been localized to numerous myenteric neurons, some submucosal neurons, and a few smooth muscle cells of guinea pig ileum (Tonini et al., 2005) and in human corneal epithelium and endothelium (Grueb et al., 2006). An upregulated expression of 5-HT_7 receptors was detected during maturation of bone marrow–derived dendritic cells, suggesting a critical role of 5-HT_7 receptors in the regulation of immune cell polarization and, thus, in the peripheral inflammatory processes (Holst et al., 2015).

D. Pharmacology

1. Agonists. Various selective 5-HT_7 receptor agonists have been identified (Table 20). The aminotetraline derivative AS-19 has a K_d of 0.6 nM at human cloned 5-HT_7 receptors, with high selectivity over all the other 5-HT receptor subtypes (>100-fold) except 5-HT_{1D} receptors (11-fold). However, AS-19 behaves as a potent partial 5-HT_7 receptor agonist (EC_{50} = 9 nM) with a maximal effect reaching 77% of that of 5-HT in a functional assay measuring cAMP stimulation in HEK293F cells overexpressing human 5-HT_7 receptors (Brenchat et al., 2009), which can complicate interpretation of arising data using tissue and in vivo preparations.

E-55888 is another potent and selective 5-HT_7 receptor agonist with high efficacy, displaying high affinity for 5-HT_7 receptors (Kd = 2.5 nM), low affinity for 5-HT_{1A} (Kd = 700 nM), and no significant affinity for the other 5-HT receptors. E-55888 behaves as a full agonist (EC_{50} = 16 nM) and increases cAMP levels in HEK293F-expressing human 5-HT_7 receptors (Brenchat et al., 2009). Another selective 5-HT_7 receptor agonist is LP-211, with high but different affinities for rat and human recombinant 5-HT_7 receptors (Kd = 0.58 and 15.0 nM, respectively) and moderate to low affinity for other 5-HT receptors, including 5-HT_{1A} (Leopoldo et al., 2008; Hedlund et al., 2010). LP-211 induces 5-HT_7–mediated relaxation of substance P-stimulated guinea pig ileum contracture (82% of the maximal effect elicited by 5-CT). However, in HEK293 cells stably expressing human 5-HT_7 receptor (Atanes et al., 2013), LP-211 displayed insurmountable antagonism of 5-CT–stimulated cAMP signaling. These results were unexpected, as LP-211 have been extensively characterized as a 5-HT_7 receptor agonist in several ex vivo and in vivo studies [for review, see Di Pilato et al. (2014)]. The authors suggested that the inhibitory effects of LP-211 are due to an irreversible stabilization of an inactive conformational state of the receptor that is tightly associated with G_s protein, independent of agonist binding (Bruheim et al., 2003; Andressen et al., 2006).

2. Antagonists. Various selective 5-HT_7 receptor antagonists have been identified (Table 20). The first was SB-258719, which displays useful 5-HT_7 receptor affinity (K_i = 31.6 nM) with 100-fold selectivity against all the other 5-HT receptors (Forbes et al., 1998). In HEK293 cells stably expressing the human 5-HT_7 receptor, SB-258719 did not stimulate basal adenylyl cyclase activity, suggesting a lack of agonist activity, but produced a surmountable antagonism of the 5-CT response with a pK_B of 7.0. Further chemical optimization of SB-258719 led to SB-269970, which is the

TABLE 20
Binding profile of the selective 5-HT_7 receptor agonists and antagonists
K_i >1 μM or percent inhibition at 1.0 μM lower than 50%.

Receptor	Affinity (K_i [nM])					
	Agonists/Partial Agonists			Antagonists		
	E-55888[a]	AS-19[a]	LP-211[b]	SB-258719[c]	SB-269970[d]	SB-656104[e]
h5-HT_7	**0.6**	**2.5**	**15**	**31.6**	**1.3**	**2.0**
h5-HT_{1A}	89.7	700	379	>7900	>10,000	562
h5-HT_{1B}	ND	ND	215	>5000	>1000	630
r5-HT_{1B}	490	n.s.	ND	ND	ND	ND
h5-HT_{1D}	6.6	n.s.	394	3162	1584	25
b5-HT_{1D}	n.s.	n.s.	ND	ND	ND	ND
h5-ht_{1e}	ND	ND	>10,000	>10,000	>6000	>5000
h5-HT_{2A}	n.s.	n.s.	626	>10,000	>10,000	63
h5-HT_{2B}	n.s.	n.s.	67	>5000	10,000	91
h5-HT_{2C}	n.s.	n.s.	ND	>10,000	10,000	269
r5-HT_{2C}	ND	ND	91	ND	ND	ND
h5-HT_3	n.s.	n.s.	>10,000	ND	ND	ND
h5-HT_4	ND	n.s.	ND	>10,000	1259	1900
h5-HT_{5A}	98.5	n.s.	178	ND	63	182
h5-HT_6	n.s.	n.s.	1571	>10,000	6300	851
h5-HT transporter	n.s.	n.s.	812	ND	ND	ND

ND, not determined; n.s., not significant.
[a]Data taken from Brenchat et al. (2009).
[b]Data taken from Hedlund et al. (2010).
[c]Data taken from Forbes et al. (1998).
[d]Data taken from Lovell et al. (2000).
[e]Data taken from Thomas et al. (2003).

prototype 5-HT_7 receptor antagonist (Hagan et al., 2000). SB-269970 displays high affinity at both human cloned 5-HT_7 receptors (pK_i = 8.9) and in guinea pig cortex (pK_i = 8.3). The compound is at least 100-fold selective against a wide range of receptors, except for the human 5-HT_{5A} receptor (about 50-fold). SB-269970 displays potent antagonism in both HEK293 cells stably expressing the 5-HT_7 receptor (pA_2 = 8.5) and in the guinea pig hippocampal membranes (pK_B = 8.3). SB-269970 also produces a small inhibition of basal adenylyl cyclase activity in the absence of added 5-CT, consistent with inverse agonism (Hagan et al., 2000). Subsequent studies led to the identification of the antagonist SB-656104, which is as potent as SB-269970 (K_i = 2 nM; pA_2 = 8.4) but displays only 10-fold selectivity over the 5-HT_{1D} receptor. Both SB-269970 and SB-656104 are brain-penetrant, and the latter is more stable metabolically (Thomas et al., 2003).

3. Allosteric Modulators. Oleamide, an amidated fatty acid, is an allosteric modulator of the 5-HT_7 receptor (Thomas et al., 1997a, 1999,b; Hedlund et al., 1999; Alberts et al., 2001). In cells expressing the 5-HT_7 receptor, oleamide increases cAMP accumulation in a concentration-dependent manner but with a lower efficacy than 5-HT. In the presence of 5-HT, oleamide displayed the opposite effect on cAMP, causing insurmountable antagonism of 5-HT, suggesting that oleamide acts at an allosteric site on the 5-HT_7 receptor. In receptor-binding studies, oleamide modulated the binding properties of 5-HT_7 receptors expressed in vitro (Hedlund et al., 1999). In addition, the binding of both agonists and antagonists at human recombinant 5-HT_7 receptors stably expressed in HEK293 cells was allosterically inhibited by zinc (Satała et al., 2018).

E. Signaling

The 5-HT_7 receptor is positively coupled to adenylyl cyclase through activation of G_s, resulting in intracellular increase in cAMP (Bard et al., 1993; Lovenberg et al., 1993a; Ruat et al., 1993b). The human, rat, and mouse splice variants all activate adenylyl cyclase (Heidmann et al., 1998; Krobert et al., 2001; Krobert and Levy, 2002; Gellynck et al., 2008) and do not show evident differences in their respective pharmacology and signal transduction properties or functional coupling to G_s protein (Heidmann et al., 1998; Krobert et al., 2001). The only reported functional difference is that the human 5-$HT_{7(d)}$ receptor displays a differential pattern of receptor internalization (Guthrie et al., 2005). Furthermore, variation in the length of the C-terminal tails and in the number of consensus sites for phosphorylation by PKA and PKC raises the possibility that the splice variants can display different desensitization or trafficking properties (Heidmann et al., 1997). Moreover, the human 5-$HT_{7(b)}$ and the rat 5-$HT_{7(b)}$ and 5-$HT_{7(c)}$ receptor splice variants contain recognition sites for PDZ domain–containing proteins (Hamblin et al., 1998), suggesting that these splice variants may be targeted to active zones within cells and may couple to alternative signaling pathways.

Stimulation of the human 5-$HT_{7(a)}$ receptor in HEK293 cells coexpressed with various adenylyl cyclase isoforms revealed that this receptor not only activates the G_s-sensitive AC5 isoform but also the G_s-insensitive

and Ca^{2+}/calmodulin-stimulated AC1 and AC8 isoforms (Nielsen et al., 1996; Xia and Storm, 1997; Baker et al., 1998).

5-HT_7 receptor expression density does not appear to influence agonist or partial agonist potency (or efficacy), suggesting the absence of receptor reserve effects (Bruheim et al., 2003; Andressen et al., 2006) and implying independence from receptor-G_s stoichiometry. This may also suggest the 5-HT_7 receptor is preassociated with G_s (Adham et al., 1998; Alberts et al., 2001; Krobert et al., 2001), which was supported by a more recent study investigating receptor-immobilized fluorescence recovery after photobleaching and fluorescence resonance energy transfer (Andressen et al., 2018) to compare the G_s coupling of the 5-HT_7 and 5-HT_4 receptors.

Prolonged stimulation by 5-HT induces both homo- and heterologous desensitization of G_s signaling in HEK293 cells. A similar effect is seen on prolonged incubation with the inverse agonists SB-269970 and methiothepin, implying that desensitization is independent of G_s activation (Krobert et al., 2006).

Clozapine and olanzapine, which are 5-HT_7 receptor inverse agonists [see Krobert and Levy (2002)], display functional selectivity at the human 5-HT_7 receptor (i.e., different ligands at the same receptor elicit different functional effects) (Andressen et al., 2015). Thus, clozapine and olanzapine downregulate 5-HT_7 receptors expressed in HEK293 cells, an effect normally evident for agonists but not for antagonists or inverse agonists. This inverse agonist–mediated receptor downregulation may be mediated by interaction with the G protein–associated sorting protein GASP-1 (Manfra et al., 2015).

In rat hippocampal neurons, 5-HT stimulation leads to the activation of serine/threonine kinases ERK1 and ERK2, and most of this activity is mediated by 5-HT_7 receptors (Errico et al., 2001). A PKA-dependent pathway for 5-HT_7 receptor–mediated ERK1 and ERK2 activation has been proposed (Norum et al., 2003, 2005). 5-HT_7 receptor activation increases intracellular cAMP levels to activate PKA, which phosphorylates the guanine nucleotide exchange factor (GEF) Ras-GRF1 that controls the activity of Ras, an upstream activator of c-Raf (or Raf-1; a member of the Raf kinase family; Norum et al., 2003). Consequently, c-Raf phosphorylates and activates various kinases, including MEK1 and, further downstream, ERK1/2. An alternative pathway could involve the activation of GEF proteins Epac1 and Epac2 by cAMP, which in turn activate the Rap1 proteins through GDP-GTP exchange. GTP-bound Rap1 proteins can bind and activate different members of the Raf kinase family (i.e., B-Raf, A-Raf, and c-Raf), leading to ERK1/2 activation (Norum et al., 2005). The contribution of these GEFs in the ERK1/2 activation pathway mediated by 5-HT_7 receptor has been demonstrated in the pheochromocytoma PC12 cells stably overexpressing 5-HT_7 receptor and in rat hippocampal neurons (Lin et al., 2003; Johnson-Farley et al., 2005). Moreover, ERK1/2 seems to be required for normal neuronal function, such as neurotrophin-stimulated neuronal differentiation and neuroprotection as well as regulation of neurite outgrowth and formation of neuronal networks (Hetman et al., 1999; Speranza et al., 2013, 2015).

Stimulation of 5-HT_7 receptor expressed in HEK293 cells increases intracellular Ca^{2+} levels (Baker et al., 1998). However, the exact mechanism for this increase is not known. In rat adrenal glomerulosa cells, this 5-HT_7 receptor–mediated increase of intracellular Ca^{2+} levels is mediated by T-type Ca^{2+} channels in a cAMP/PKA-dependent manner (Lenglet et al., 2002a,b), which is likely to contribute to ERK1/2 activation (Lin et al., 2003; Norum et al., 2003; Johnson-Farley et al., 2005).

The 5-HT_7 receptor interacts with the G_{12} member of the G protein family (Kvachnina et al., 2005; Kobe et al., 2012), which can activate multiple signaling pathways. The prominent downstream effectors are members of the Rho family of small GTPases (Rho, Rac, and Cdc42). 5-HT_7 receptor–mediated stimulation of G_{12} protein results in Rho-dependent activation of the transcription factor serum response factor, which binds to the serum response element (SRE). The stimulation of 5-HT_7 receptor increases SRE-driven gene expression even in the presence of a PKA inhibitor or pertussis toxin, suggesting that the receptor-mediated SRE activation is not PKA-dependent (Kvachnina et al., 2005). Stimulation of 5-HT_7 receptor/G_{12} signaling pathway selectively activates both RhoA and Cdc42 (Kvachnina et al., 2005), suggesting a cross talk between Cdc42 and RhoA pathways. The 5-HT_7 receptor/G_{12} signaling pathway in cultured hippocampal neurons promotes formation of dendritic spines and accelerates synaptogenesis, leading to enhanced spontaneous synaptic activity (Kobe et al., 2012). A morphogenic action of the 5-HT_7 receptor is confirmed in neuronal primary cultures from the cortex, hippocampus, and striatal complex of embryonic rat or mouse brain (Speranza et al., 2013, 2015) and postnatal cortical and striatal neurons (Speranza et al., 2017). The involvement of mTOR, Cdc42, Cdk5, and ERK in this process suggests these proteins to be downstream targets of G_{12} (Speranza et al., 2013, 2015). In the hippocampus, the 5-HT_7 receptor/G_{12} signaling pathway undergoes strong developmental regulation. In organotypic hippocampal cultures from juvenile mice, G_{12} signaling potentiates formation of dendritic spines, increases the basal neuronal excitability, and modulates synaptic plasticity. In contrast, in preparations from older mice, 5-HT_7 receptor stimulation had no effect on neuronal morphology, synaptogenesis, and synaptic plasticity (Kobe et al., 2012). Consistently, the expression level of both 5-HT_7 receptor and G_{12} proteins in the hippocampus

progressively decreases during postnatal development (Kobe et al., 2012).

F. Post-translational Modifications

1. Regulatory Mechanisms. The 5-HT_7 receptor appears to undergo *N*-glycosylation and palmitoylation. The receptor is *N*-glycosylated at the asparagine residues N5 and N66 (Gellynck et al., 2012), but this does not appear to influence agonist binding, potency, or efficacy. Furthermore, immunocytochemical studies revealed the presence of the *N*-glycosylation mutants at the cell surface.

The mouse 5-HT_7 receptor expressed in Sf9 insect cells undergoes dynamic palmitoylation in an agonist-dependent manner (Kvachnina et al., 2009). Mutation analysis shows that cysteines located in the C-terminal receptor domain at positions 404, 438, and 441 represent the main potential palmitoylation sites. Palmitoylation-deficient mutants reveal that agonist-induced activation of G_s and G_{12} proteins is unaffected. Instead, mutation of the Cys404 alone or in combination with Cys438/Cys441 increases G_s-mediated constitutive activity of the 5-HT_7 receptor (agonist-independent), whereas the activation of G_{12} protein is not affected. Thus, palmitoylation of 5-HT_7 receptors might be directly involved in the isomerization of the receptor from the inactive to the active form in the absence of agonists. Considering that the 5-HT_7 receptor is coupled to both G_s and G_{12} proteins, dynamic palmitoylation may represent a molecular mechanism responsible for selective G_s- or G_{12}-mediated signaling.

2. Interacting Proteins. Apart from G proteins, only a few 5-HT_7 receptor–interacting proteins have been described. Interacting proteins mostly act as adaptor or scaffolding proteins and help in trafficking of the receptor, not only to the plasma membrane but also in receptor internalization, recycling, and degradation. Among the many regulatory proteins that are involved in GPCR desensitization and downregulation, GPCR-associated sorting proteins (GASPs) participate in the sorting of several receptors toward the degradation pathway (Bornert et al., 2013). GASP-1 and GASP-2 interact with several GPCRs, including 5-HT_7 receptors, through interaction of C-terminal tails containing two critical amino acid residues within the sequence that corresponds to helix 8 in the three-dimensional structure of rhodopsin (Simonin et al., 2004).

PLAC-24 (protein that localizes at cell-cell contacts), also known as the eukaryotic initiation of translation factor 3, subunit k, is another intracellular interaction partner for all three splice variants of the human 5-HT_7 receptor (De Martelaere et al., 2007). PLAC-24 might be involved in the transport of newly synthesized 5-HT_7 receptors toward the plasma membrane, as this transport is hampered by the overexpression of certain domain constructs of PLAC-24. PLAC-24 has been proposed to be additionally involved in the stabilization of the receptor at the cell surface by anchoring it at the actin cytoskeleton, as PLAC-24 might be part of a multisubunit complex that links the actin and microtubule cytoskeleton and causes an increase in the expression of the receptor.

Periplakin (an actin- and intermediate filament–binding protein) and the neurite-outgrowth promoting protein, neurochondrin, strongly interact with the C-terminal tail of 5-HT_7 receptor. The functional consequences of these specific interactions are not yet known (Ward et al., 2009).

RhoBTB3, a member of the Ras superfamily of small GTPases, is expressed in several brain regions where the 5-HT_7 receptor is also localized, including hippocampus and nucleus accumbens. The physical interaction between the 5-HT_{7a} receptor and RhoBTB3 has been demonstrated through yeast two hybrid, GST pull-down, and coimmunoprecipitation assays (Matthys et al., 2012). Not only the C-terminal tail but also the third intracellular loop of the 5-HT_{7a} receptor seem to be involved in the interaction. The 5-HT_{7a} receptor may be targeted for proteasomal degradation following endocytosis at the plasma membrane, and RhoBTB3 appears to strongly inhibit proteasomal degradation of the receptor.

S100B is a Ca^{2+}-regulatory protein that controls Ca^{2+} homeostasis in various cell types, including astrocytes. S100B also regulates the activity of adenylyl cyclase in preparations from rat brain and skeletal muscle (Donato et al., 2009). S100B physically interacts with 5-HT_7 receptors; it negatively regulates cAMP accumulation in 5-HT_7–transfected HeLa cells and mouse cortical astrocytes. Overexpression of S100B causes brain region–specific dysregulation of cAMP pathways in vivo that may relate to depressive-like behavior, which can be normalized by 5-HT_7 receptor blockade by SB-269970 (Stroth and Svenningsson, 2015).

For further discussion of interacting proteins, see *XVII. B. A Survey of 5-HT Receptor GIPs*.

3. Homo- and Heteromeric Receptor Associations. GPCRs can form oligomers, and it is now widely accepted that homo- and heterodimerization provides an additional mechanism for regulating cellular processes through the fine tuning of receptor-mediated signaling (Devi, 2001; Bulenger et al., 2005). The 5-HT_7 receptor forms homodimers in both intact HEK293 cells and neuroblastoma N1E-115 cells transfected with 5-HT_7 receptor and rat cortical astrocytes (Teitler et al., 2010; Smith et al., 2011; Renner et al., 2012; Teitler and Klein, 2012).

Heterodimerization between 5-HT_{1A} and 5-HT_7 receptors has been demonstrated by coimmunoprecipitation and by fluorescence resonance energy transfer approaches (Renner et al., 2012); the coimmunoprecipitation studies in mouse brain provide direct evidence that 5-HT_{1A} and 5-HT_7 receptors can form heterodimers in vivo. Such heterodimerization alters the signaling properties of the 5-HT_{1A} receptor by attenuating the ability of 5-HT_{1A}

receptor to activate G_i protein, in contrast to 5-HT_7 receptor–mediated activation of Gs protein, which is not affected. In addition, heterodimerization reduces the ability of 5-HT_{1A} receptors to activate GIRK channels, an effect mediated through the $G\beta\gamma$ subunits of G_i proteins (Reuveny et al., 1994; Kofuji et al., 1995). The inhibitory effect of heterodimerization on GIRK currents is also evident in mouse hippocampal neurons, suggesting a physiologic relevance in vivo.

Heterodimerization between the 5-HT_{1A} and 5-HT_7 receptors appears to promote agonist-mediated internalization of the 5-HT_{1A} receptor (5-HT_{1A} receptors expressed alone are relatively resistant to the agonist-induced internalization). The pharmacological blockade of 5-HT_7 receptors, but not of 5-HT_{1A} receptors, abolishes internalization of both 5-HT_7 homo- and heterodimers, suggesting that 5-HT_7 receptor–mediated signaling is an initial step responsible for 5-HT_{1A} receptor cointernalization. Once internalized, 5-HT_{1A} receptors can activate G protein–independent signaling pathways such as a β-arrestin–mediated coupling to MAPK. Thus, depending on the relative amount of 5-HT_{1A} receptors participating in dimers, stimulation by 5-HT can activate distinct ERK-mediated pathways (i.e., G protein–dependent or β-arrestin–dependent), further implicating the physiologic relevance of heterodimerization.

G. Function

Hedlund et al. (2003) first reported on the generation of 5-HT_7 KO mice by targeted disruption within exon II of the 5-HT_7 receptor gene, thus inactivating all known splice variants of the receptor. 5-HT_7 KO mice (Roberts et al., 2004a; Guscott et al., 2005) grow and reproduce, normally suggesting that the receptor does not play an essential role during development. These mice also have normal body weight and basal rectal temperature and appear to be in good health (Guscott et al., 2005). No differences are detected between 5-HT_7 KO mice and the wild-type littermates in general motor ability, visual acuity, pain sensitivity, anxiety-like behavior, or the capacity to show freezing behavior in the habituation, conditioning, or cued components of the cued and contextual conditioning procedure (Roberts et al., 2004a). Also, in the prepulse inhibition paradigm, no difference is observed in the 5-HT_7 receptor KO mice (Guscott et al., 2005; Semenova et al., 2008).

1. Thermoregulation. 5-HT_7 agonists induce a considerable hypothermic response in vivo in various species (Yamada et al., 1988; Won and Lin, 1988; Sugimoto et al., 1991; Guscott et al., 2003; Hedlund et al., 2003, 2004; Naumenko et al., 2011). Initially, the 5-HT_{1A} receptor was thought responsible, largely on the basis of data obtained using the 5-HT_{1A} receptor agonist 8-OH-DPAT (Hjorth, 1985) before this drug was also recognized as a 5-HT_7 receptor agonist (Guscott et al., 2003; Hedlund et al., 2003, 2004). It appears the 5-HT_7 receptor is more important at lower 5-HT concentrations, playing a role in the fine tuning of temperature homeostasis, whereas the 5-HT_{1A} receptor is activated at higher 5-HT concentrations, possibly as a defense against hyperthermia (Hedlund et al., 2004), such that both 5-HT_{1A} and 5-HT_7 receptors are important (Brenchat et al., 2012a).

2. Learning and Memory. 5-HT_7 receptor KO mice under certain types of examination can display specific impairments in contextual learning. Two forms of place learning, spatial (Barnes maze) and contextual (fear conditioning), in addition to three hippocampus-dependent learning tests have been studied (motor, cued conditioning, and operant conditioning) to demonstrate effects of 5-HT_7 receptor KO mice in contextual fear conditioning, whereas there was no evident effect in the other learning tests. Of potential relevance, electrophysiological studies on hippocampal slices from 5-HT_7 receptor KO mice demonstrate deficits in long-term potentiation (Roberts et al., 2004).

Further behavioral studies demonstrate hippocampus-associated spatial memory deficits in 5-HT_7 receptor KO mice (impairments in memory compilation required for resolving spatial tasks), which result in impaired hippocampus-dependent allocentric memory (Sarkisyan and Hedlund, 2009), whereas no effect was evident in the novel object recognition test. Egocentric spatial memory, which is striatum-dependent, remains intact. On the other hand, 5-HT_7 receptor KO mice do not exhibit learning impairments and/or dysfunctions in short-term memory if the environment remains static, such as in the Barnes maze test. In the same test, 5-HT_7 receptor KO mice have no impairment in long-term memory or memory consolidation.

3. Antipsychotic Potential. 5-HT_7 receptor antagonists have been evaluated in animal models used to test antipsychotic-like activity. The selective 5-HT_7 receptor antagonist SB-258741 reverses hyperactivity induced by PCP in rats (Pouzet et al., 2002). SB-269970 partially but significantly blocks hyperactivity induced by ketamine in mice (Galici et al., 2008). Furthermore, 5-HT_7 receptor KO mice display less pronounced deficits in the PCP-induced prepulse inhibition compared with WT mice (Semenova et al., 2008), although this effect was not replicated by SB-269970 treatment (Semenova et al., 2008). Collectively, the available data suggest that the antipsychotic-like activity elicited by selective 5-HT_7 receptor blockade is weaker than that obtained with clinically proven antipsychotic drugs (Thomas and Hagan, 2004).

Current antipsychotic drugs are not very effective to reverse the cognitive deficits associated with schizophrenia. Cognitive impairments induced by subchronic PCP administration in rats are believed to mimic cognitive deficits in schizophrenia (Javitt and Zukin, 1991; Jentsch and Roth, 1999). PCP selectively impairs performance in reversal learning test (Abdul-Monim et al., 2006, 2007; McLean et al., 2009a), attentional set-shifting test (McLean et al., 2008), and novel object

recognition test (a paradigm for studying visual episodic memory) (Grayson et al., 2007). The acute administration of SB-269970 reverses subchronic PCP-induced deficits in a reversal learning task in rats (McLean et al., 2009b) and in the novel object recognition test in rats (Horiguchi et al., 2011). Pretreatment with SB-269970 or lurasidone reverses the subchronic PCP-induced deficit in reversal learning in mice (Rajagopal et al., 2016). This effect was not elicited by the agonist AS-19, confirming that antagonism, but not agonism, at 5-HT_7 receptors restores function in principal cortical neurons impaired by NMDA receptor blockade.

Cognitive deficits in mice induced by dizocilpine are also used to model impaired working memory in schizophrenia. SB-269970 reverts dizocilpine-induced cognitive deficits in a translational behavioral model of working memory, the delayed nonmatching to position task. At a neurochemical level, SB-269970 normalizes the dizocilpine-induced glutamate efflux but not dizocilpine-induced dopamine extracellular levels in the cortex of freely moving rats (Bonaventure et al., 2011). SB-269970 also reverses dizocilpine-induced memory deficits in an autoshaping Pavlovian instrumental learning task in rats (Meneses, 2004). Moreover, 5-HT_7 receptor blockade by another antagonist, SB-656104, reverses dizocilpine-induced learning and memory impairments in the passive avoidance and Morris water maze tests in rats (Horisawa et al., 2011).

Ketamine-based animal models represent a valuable tool in preclinical research because ketamine is commonly used in the clinic to model the transient neurocognitive impairments in healthy volunteers (Krystal et al., 1994). Acute administration of SB-269970 in rats ameliorates ketamine-induced cognitive deficits in the attentional set-shifting task (a measure of cognitive flexibility) and the novel object recognition test (Nikiforuk et al., 2013). SB-269970 reverses memory deficits in an autoshaping Pavlovian instrumental learning task in rats after an intraprefrontal infusion of ketamine (Liy-Salmeron and Meneses, 2008).

Experimental evidences suggest a role of 5-HT_7 receptor blockade in the procognitive actions of the atypical antipsychotics amisulpride and lurasidone. Both drugs ameliorate the PCP-induced deficits in the novel object recognition task in rats (Horiguchi et al., 2011). This effect is reversed by coadministration of the selective 5-HT_7 agonist AS-19. Lurasidone attenuates the dizocilpine-induced deficits in the passive avoidance test in rats. Also, in this case, AS-19 abolishes the effect of lurasidone (Horisawa et al., 2013).

The pharmacological blockade of 5-HT_7 receptors may also have therapeutic implications for the treatment of negative symptoms in schizophrenia because SB-269970 ameliorates ketamine-induced social withdrawal in rats (Nikiforuk and Popik, 2013). Consistently, acute administration of amisulpride reverses ketamine-induced social withdrawal. The prosocial efficacy of amisulpride is abolished by the agonist AS-19. Finally, coadministration of subactive doses of SB-269970 and amisulpride results in prosocial effects in rats (Hołuj et al., 2015).

4. Antidepressant-Like Behavior. 5-HT_7 receptor KO mice have an antidepressant-like behavior, with reduced immobility in commonly used preclinical animal models of depression, such as the tail suspension test (TST) and the forced swim test (Guscott et al., 2005; Hedlund et al., 2005; Sarkisyan et al., 2010). The decreased immobility in both models is most likely not due to a general increase in motor activity, as no genotype difference in locomotor activity or motor learning were evident between 5-HT_7 receptor KO and WT mice (Roberts et al., 2004).

Because 5-HT_7 receptor KO mice display an antidepressant-like behavior, the potential role of the 5-HT_7 receptor was also investigated in OCD models: it is recognized OCD involves the 5-HT system and patients may benefit from antidepressant therapy. Thus, 5-HT_7 receptor KO mice have been tested in three models believed to mimic some of the stereotypic aspects of OCD (Hedlund and Sutcliffe, 2007). Inactivation of the 5-HT_7 receptor leads to decreased marble burying, a model linked to OCD and anxiety. However, there is no difference between 5-HT_7 receptor KO and WT mice in the two other models (i.e., head dipping and plastic-mesh screen chewing models). Thus, a possible role of the receptor in OCD remains an open question.

5. Sleep. Overall, a normal sleep pattern is observed in 5-HT_7 receptor KO mice, although during rest, 5-HT_7 receptor KO mice spend less time in REM sleep (with less frequent but longer REM episodes) compared with WT mice (Hedlund et al., 2005). There is no difference between the genotypes in time spent awake or in slow wave sleep, and the frequency of slow wave sleep episodes is not altered. These sleep patterns of 5-HT_7 receptor KO mice are in agreement with the antidepressant-like profile observed in this genotype.

A variety of circadian parameters in 5-HT_7 receptor KO mice, including rate of entrainment and photic responsiveness, have been investigated. There are no evident differences in the average number of days that 5-HT_7 receptor KO mice need to reach entrainment to an advance of 6 hours in the light/dark cycle compared with WT mice (Gardani and Biello, 2008). Both groups of mice display minimal effects to light stimulation during the subjective day, whereas, during the early night, light induces phase delays and, later in the subjective night, results in advances of the circadian phase.

Similarly, administration of the 5-HT_7 receptor antagonists SB-269970 and SB-656104 to rats reduces the total amount of REM sleep, whereas wake and slow wave sleep are not affected (Hagan et al., 2000; Thomas et al., 2003; Monti and Jantos, 2006). Furthermore, both

genetic inactivation and pharmacological blockade of 5-HT_7 receptor augmented the effects of SSRIs on REM sleep suppression (Bonaventure et al., 2007; Shelton et al., 2009).

As for the effect of 5-HT_7 receptor agonists on sleep, systemic administration of LP-211 during the light phase increases wakefulness in rats and reduces REM sleep duration and periods. To assess the potential neural sites that mediate the changes in REM and wake in the rat, LP-211 was microinjected into the brain regions involved in sleep-wake regulation. Local administration of LP-211 into the DRN, locus coeruleus, lateral basal forebrain, and laterodorsal tegmental nucleus suppressed REM sleep, and microinjection of LP-211 into the basal forebrain augmented wake (Monti et al., 2014). The authors proposed that activation of 5-HT_7 receptors expressed by GABAergic interneurons decreases the activity of REM sleep–promoting cholinergic neurons in the laterodorsal and pedunculopontine tegmental nuclei and reduces REM sleep. Suppression of REM sleep was observed also when LP-44, another 5-HT_7 receptor agonist, was microinjected into the DRN (Monti et al., 2008).

A possible explanation for these apparently contradictory findings is that 5-HT_7 receptors can stimulate GABAergic neurons only in the absence and/or at low concentrations of 5-HT, whereas at high 5-HT concentrations, 5-HT_7 receptors inhibit these GABAergic neurons. Therefore, a concentration-dependent switch in 5-HT_7 receptor signaling could explain why inhibition of 5-HT_7 receptor activity, elevated 5-HT concentrations, or administration of 5-HT_7 receptor agonist at high concentrations prevent 5-HT_7 receptor–mediated stimulation of the REM stimulatory GABAergic neurons.

5-HT_7 receptors have also been implicated in the regulation of the mammalian circadian clock located in the suprachiasmatic nucleus. Studies have demonstrated that 8-OH-DPAT induces nonphotic phase resetting through activation of 5-HT_7 receptors in vitro and in vivo. This effect is reversed by genetic inactivation or pharmacological blockade by the antagonists SB-269970, DR-4004, and JNJ-18038683 (Sprouse et al., 2004; Guscott et al., 2005; Shelton et al., 2015). Consistently, the agonist LP-211 induced a phase advancement of the circadian rhythm in mice (Adriani et al., 2012). Moreover, activation of 5-HT_7 receptors by the partial agonist AS19 shortens the period length of oscillation of clock gene period circadian protein homolog 2 expression in the suprachiasmatic nucleus. Period circadian protein homolog 2 expression is used to monitor changes in circadian period length and amplitude (Westrich et al., 2013).

6. Autism Spectrum Disorders. Clinical studies suggest a deficient brain 5-HT system as a causal mechanism in autism spectrum disorders (McDougle et al., 1996; Boccuto et al., 2013). Moreover, the lack of 5-HT during early stages of development may contribute to disrupt the wiring architecture of the brain (Azmitia et al., 2011).

5-HT_7 receptor activation corrects molecular, electrophysiological, and behavioral manifestations in mice models of Fragile X syndrome (FXS) and Rett syndrome (RTT), both genetic forms of intellectual disabilities associated with autistic behavior (Costa et al., 2012, 2015; De Filippis et al., 2014, 2015). FXS is the most common inherited intellectual disability; it is caused by silencing of the Fmr1 gene coding for the Fragile X Mental Retardation Protein (Pieretti et al., 1991).

Activation of 5-HT_7 receptors by the agonists 8-OH-DPAT and LP-211 rescues mGluR-LTD in Fmr1 knockout mice and restores LTD levels to those of WT mice (Costa et al., 2012, 2015). This might have important functional consequences, as long-term synaptic plasticity plays a fundamental role in shaping the structure and function of brain circuits. LTD is crucially involved in learning and memory, in novelty detection, and in the extinction of previously acquired memories, and it is believed to underlie behavioral flexibility (Collingridge et al., 2010). On that basis, Costa et al. (2012, 2015) suggested that selective activation of 5-HT_7 receptors, by restoring mGluR-mediated synaptic plasticity to normal levels, might also rescue cognitive functions and behavioral flexibility in the mouse model of FXS.

De Filippis et al. (2014) have demonstrated that the selective 5-HT_7 agonist LP-211 rescues the behavioral impairments in MeCP2-308 male mice, a mouse model of RTT. RTT is a rare neurodevelopmental disorder characterized by severe behavioral symptoms, including autistic-like behaviors, anxiety, motor disturbances, stereotypic hand movements, and severe cognitive dysfunction (Hagberg, 2002; Ricceri et al., 2013). Mutations in the methyl-CpG–binding protein 2 (MeCP2) gene have been identified as the main genetic cause of RTT. MeCP2 encodes a multifunctional protein that binds to methylated DNA and mainly acts as a key transcriptional regulator. Systemic treatment with LP-211 rescues RTT-related defective performance: anxiety-related profiles in a light/dark test, motor abilities in a dowel test, the exploratory behavior in the marble burying test, and memory in the novelty preference task. At a molecular level, LP-211 administration in MeCP2-308 mice restores levels of the Rho GTPases effector molecules p21 activated kinases and cofilin, which both are key regulators of actin cytoskeleton dynamics and, thus, crucially involved in neuronal plasticity. A follow-up study reported similar effects in MeCP2-308 heterozygous female mice; the genetic and hormonal milieus of these mice more closely resemble those of RTT patients (De Filippis et al., 2015). In addition, targeting 5-HT_7 receptors can rescue brain mitochondrial dysfunction in heterozygous female MeCP2-308 and MeCP2-Bird mice (a more severely affected model). Moreover, LP-211 treatment

completely restores the radical species overproduction by brain mitochondria in the MeCP2-308 model and partially recovers the oxidative imbalance in MeCP2-Bird mice (Valenti et al., 2017).

A core symptom of autism spectrum disorder is repetitive and stereotypic behavior. The dual 5-HT_{1A}/5-HT_7 partial agonist (+)-5-(2'-fluorophenyl)-N,N-dimethyl-1,2,3,4-tetrahydronaphthalen-2-amine reduces or eliminates stereotypy in three different mouse models of stereotypy: idiopathic jumping, repetitive body rotations after treatment with the NMDA antagonist dizocilpine, and repetitive head-twitching after treatment with the 5-HT_2 receptor agonist DOI (Canal et al., 2015) without altering locomotor behavior.

Finally, it has been proposed that the reduced behavioral inflexibility elicited by 5-HT_7 receptor antagonists (Nikiforuk, 2012; Nikiforuk and Popik, 2013) might be of relevance in autism spectrum disorder, as reduced behavioral flexibility (i.e., a reduced ability to replace a previously acquired rule with a new one in adaptation to a new environmental context) is a typical feature of this pathology (Ciranna and Catania, 2014).

There is a suggestive link between a *HTR7* genetic abnormality, which encodes the 5-HT_7 receptor, and neurodevelopmental disorders (Helsmoortel et al., 2016). The whole-genome sequencing of a severely affected dizygotic twin with an autism spectrum disorder and intellectual disability revealed a compound heterozygous mutation in the *HTR7* gene as the only variation.

7. Epilepsy. The involvement of 5-HT_7 receptors in epilepsy has been actively investigated. The 5-HT_7 receptor antagonist SB-258719 reduces spontaneous epileptic activity in the WAG/Rij rat model of absence epilepsy (Graf et al., 2004). In pilocarpine-induced rat models of temporal lobe epilepsy, SB-269970 also reduces the number of seizures (Yang et al., 2012). Consistently, in epileptic rats, the presumed activation of 5-HT_7 receptors by AS-19 increases the number of seizures (Yang et al., 2012). In contrast to the above data, inactivation of the 5-HT_7 receptor gene caused a generalized threshold reduction in electrical- or chemical-induced seizures (Witkin et al., 2007).

It has been suggested that the evident seizure-prone phenotype of 5-HT_7 receptor KO mice may be due to adaptive changes to the loss of perinatal 5-HT_7 receptor–induced depolarization. In line with these data, the 5-HT_7/5-HT_{1A} receptor agonist 5-CT increased the seizure threshold for picrotoxin in stressed mice (Pericic and Svob Strac, 2007), which is abolished by SB-269970 but not by the 5-HT_{1A} receptor antagonist WAY-100635 (Pericic and Svob Strac, 2007).

8. Drug Abuse. The link between the 5-HT_7 receptor and substance abuse has been under explored. Hauser et al. (2015) suggest that 5-HT_7 receptors may play a key role in addiction on the basis of neuroanatomical, biochemical, physiologic, and behavioral observations.

Interestingly, genomic studies in humans suggest a link between variants in the gene encoding the 5-HT_7 receptor and alcoholism (Zlojutro et al., 2011; Kim et al., 2014). In this respect, mice exposed to alcohol vapors present increased expression of 5-HT_7 receptors in brain areas specifically involved in dependence (Yoshimoto et al., 2012). However, blockade of 5-HT_7 receptors with SB-258719 does not alter alcohol drinking behavior in the mice exposed to alcohol vapor (Yoshimoto et al., 2012).

9. Pain. Much effort has been made to investigate the potential (patho)physiologic role of 5-HT_7 receptors in nociception and chronic pain. Early studies suggested a peripheral pronociceptive action of 5-HT through 5-HT_7 receptor activation (Meuser et al., 2002). The pain-promoting effect of 5-HT or 5-CT injection into a hindpaw on formalin-induced local nociceptive responses is blocked by SB-269970 (Rocha-González et al., 2005).

In rat models of neuropathic pain (i.e., chronic constriction injury to the sciatic nerve or spinal nerve ligation) systemic administration of SB-269970 reduces hyperalgesia and tactile allodynia (Amaya-Castellanos et al., 2011; Viguier et al., 2012). Intrathecal administration of SB-269970 also reduces tactile allodynia in spinal nerve–ligated rats, suggesting the involvement of 5-HT_7 receptors in pronociceptive mechanisms at the spinal level (Amaya-Castellanos et al., 2011). A pronociceptive effect of 5-HT_7 receptor stimulation has been reported at the trigeminal level. SB-656104 significantly decreases the c-Fos immunostaining in the spinal nucleus of the trigeminal nerve in response to intracisternal injection of capsaicin (Martínez-García et al., 2011).

However, such findings appear in contradiction with the data from Brenchat et al. (2009, 2010), in which systemic administration of SB-269970 or SB-258719 enhance mechanical hypersensitivity associated with capsaicin-induced hyperalgesia or nerve injury in mice. Interestingly, local injection of SB-269970 or SB-258719 in control mice does not promote hypersensitivity, suggesting that 5-HT_7 receptors might be involved in some pronociceptive modulatory mechanisms only under neuronal sensitization conditions.

In addition to possible species differences, the selected model of neuropathic pain might also have relevance with respect to the aforementioned contradictions.

The antinociceptive potential of 5-HT_7 receptor antagonists would suggest a pronociceptive effect of 5-HT_7 receptor agonists for which there is some support (Brenchat et al., 2010; Martínez-García et al., 2011). However, other studies report 5-HT_7 receptors mediate antinociceptive effects. Thus, blockade of spinal 5-HT_7 receptors by intrathecal injection of SB-269970 prevents the antinociceptive effects of systemic administration of morphine, tramadol, or cannabinoids in the tail flick test (Dogrul and Seyrek, 2006; Dogrul et al., 2009; Yanarates

et al., 2010), and intrathecal administration of E-57431 and E-55888 inhibit mechanical hypersensitivity caused by capsaicin injection or nerve injury–induced mechanical hypersensitivity in both mice and rats (Brenchat et al., 2011; Viguier et al., 2012). Furthermore, systemic treatment with 5-HT_7 receptor agonists produce marked reductions in mechanical and thermal hypersensitivity in various chronic pain models with central and/or peripheral sensitization (Brenchat et al., 2009, 2010, 2012a,b; Ulugol et al., 2012; Viguier et al., 2012, 2013).

H. Clinical Relevance

1. Depression. Multiple experimental approaches tend to support the hypothesis that 5-HT_7 receptor blockade or genetic inactivation displays an antidepressant-like activity (Guscott et al., 2005; Hedlund et al., 2005; Sarkisyan et al., 2010). In agreement with mouse KO data, the pharmacological blockade of 5-HT_7 receptors results in antidepressant-like effects in the TST (Hedlund et al., 2005; Wesołowska et al., 2006a; Bonaventure et al., 2007) and in the FST in both mice (Hedlund et al., 2005; Wesołowska et al., 2006b) and rats (Wesołowska and Kowalska et al., 2008; Mnie-Filali et al., 2011).

The 5-HT_7 receptor antagonist SB-269970 was assessed in the olfactory bulbectomy paradigm, which is considered as a "chronic" behavioral model of depression, in which classic antidepressants require the administration for 2 to 3 weeks before any antidepressant-like effects can be observed (Song and Leonard, 2005). SB-269970 induced a faster antidepressant-like response when compared with 1 week of treatment with the SSRI fluoxetine (Mnie-Filali et al., 2011). 5-HT_7 receptor blockade may also augment the effects of antidepressant drugs; thus, the combination of an ineffective dose of SB-269970 with an ineffective dose of one of several antidepressants, results in a synergistic reduction in immobility in both FST and TST (Wesołowska et al., 2006b; Bonaventure et al., 2007; Wesołowska and Kowalska, 2008; Sarkisyan et al., 2010) that correlates with increases in 5-HT release in the frontal cortex (Bonaventure et al., 2007; Wesołowska and Kowalska, 2008). Besides the prefrontal cortex, the hippocampus has also been implicated in the effects of SB-269970 and imipramine, as intrahippocampal SB-269970 administration reduces immobility in the rat FST (Wesołowska et al., 2006b).

Preclinical tests in mice suggest that the clinically established antidepressant effect of the atypical antipsychotic drugs amisulpride, aripiprazole, or lurasidone may be due to 5-HT_7 receptor blockade. Thus, amisulpride, aripiprazole, and lurasidone are potent but nonselective 5-HT_7 receptor antagonists that have clear antidepressant actions (Lecrubier et al., 1997; Smeraldi, 1998; Lawler et al., 1999; Shapiro et al., 2003; Nakamura et al., 2009; Ishibashi et al., 2010; Citrome, 2011) that reduce immobility in the TST and the FST in WT but not in 5-HT_7 KO mice (Abbas et al., 2009a; Sarkisyan et al., 2010; Cates et al., 2013), strongly suggesting a role for the 5-HT_7 receptor.

The 5-HT_7 receptor antagonist JNJ-18038683 (Bonaventure et al., 2012) reduces immobility in mice in the TST. Coadministration of subeffective doses of citalopram and JNJ-18038683 elicits an antidepressant effect. However, JNJ-18038683, when tested in patients with major depressive disorder, produced no statistically significant improvement over placebo on the Montgomery-Åsberg Depression Rating Scale, although escitalopram in that same study was also inactive, complicating the interpretation.

2. Sleep. The potent and selective 5-HT_7 receptor antagonist JNJ-18038683 prolonged REM latency and decreased REM sleep duration in healthy volunteers (Bonaventure et al., 2012). Furthermore, JNJ-18038683 appeared to enhance REM sleep suppression induced by citalopram.

XVI. High-Resolution Structure of 5-HT Receptors

A. 5-HT GPCRs

Since the initial cloning of a 5-HT receptor in 1988 (Julius et al., 1988), it has been appreciated that the G protein–coupled 5-HT receptors would have a topology similar to other members of the GPCR superfamily (Kroeze et al., 2003). These features included a predicted seven-transmembrane helical arrangement with an orthosteric binding pocket near the upper one-third of the helical array (Choudhary et al., 1992, 1993, 1995). Indeed, early molecular models—bolstered by site-directed mutagenesis studies—predicted that 5-HT and serotonergic drugs such as ergolines and ergopeptines would be anchored by a highly conserved aspartic acid in helix III and aromatic residues in helix VI (Choudhary et al., 1993, 1995; Wang et al., 1993; Sealfon et al., 1995).

These predictions were confirmed in 2013 with the publication of the first high-resolution structures of the human 5-HT_{1B} (Wang et al., 2013) and 5-HT_{2B} (Wacker et al., 2013) receptors—both in complex with the ergopeptine ergotamine (Fig. 28). Additionally, the 5-HT_{1B} receptor was also solved in complex with dihydroergotamine (Wang et al., 2013), revealing an essentially identical ligand orientation. Perhaps not surprisingly, and as predicted many years ago (Choudhary et al., 1995), ergotamine was anchored by the highly conserved TMIII aspartic acid residue and stabilized by hydrophobic and edge-on-face interactions with aromatic residues in helix VI (Wacker et al., 2013; Wang et al., 2013).

Given the large differences between 5-HT_{1B} and 5-HT_{2B} receptors in terms of pharmacology, signal transduction, and amino acid sequence, the high-resolution structures of both receptors allowed the investigators to

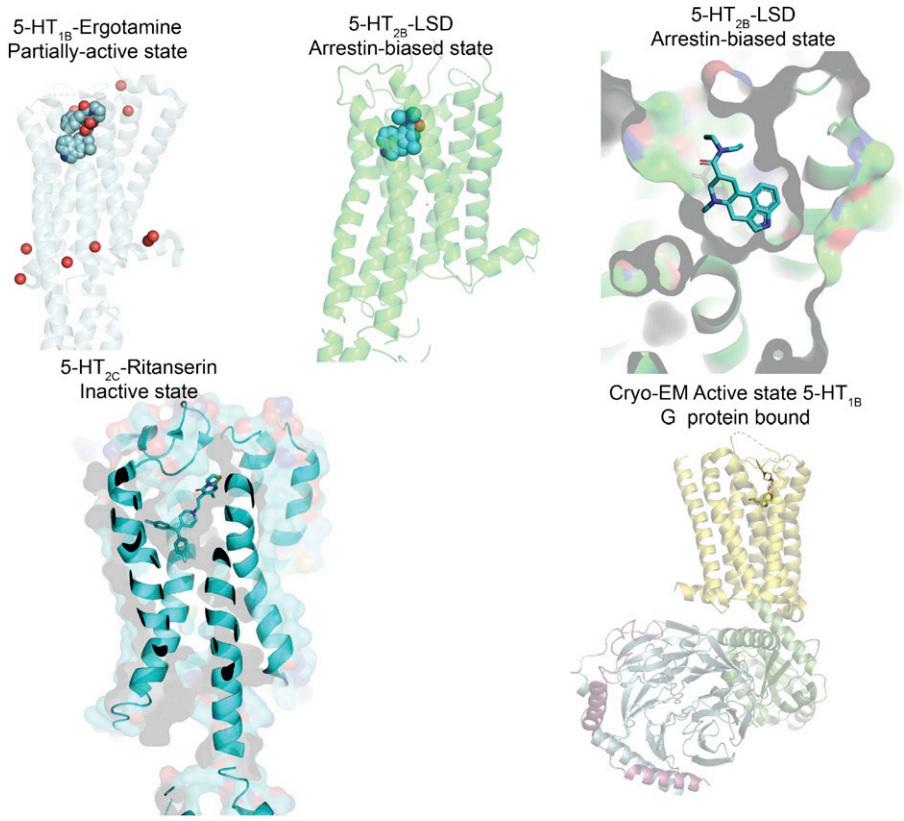

Fig. 28. Structures of 5-HT_{1B}, 5-HT_{2B}, and 5-HT_{2C} receptors. PyMol renderings of the 5-HT_{1B} receptor in complex with ergotamine (Left: Wang et al., 2013; Wacker et al., 2013) and the 5-HT_{2B} receptor in complex with LSD (Wacker et al., 2017) demonstrating different binding pockets for ergotamine and LSD [see Wacker et al. (2017) for details]. The figure also shows inactive-state structure of the 5-HT_{2C} receptor (Peng et al., 2018) and the G protein–coupled state of the 5-HT_{1B} receptor (Garcia-Nafria et al., 2018)

identify both important structural commonalities and dissimilarities between these two distinct 5-HT receptor families (Wacker et al., 2013; Wang et al., 2013); the orthosteric binding pockets of both receptors are nearly identical, with only two amino acids in the conserved orthosteric binding site differing (Wang et al., 2013). This high degree of conservation readily explains why many serotonergic drugs are promiscuous. Indeed, LSD was found to be a potent agonist at every G protein–coupled 5-HT receptor except the 5-HT_7 (Wacker et al., 2013).

Subtle differences in the general region of the binding pocket were able to explain heretofore puzzling aspects of 5-HT receptor pharmacology. For instance, the apparent preference of rodent versions of the 5-HT_{1B}, 5-HT_{1D}, 5-ht_{1e}, and 5-HT_{1F} receptors for β-adrenergic antagonists (Hoyer and Middlemiss, 1989; Adham et al., 1994) was now apparent based on structural, mutagenesis, and modeling studies (Wang et al., 2013). Thus, a $Thr^{7.39}Asn^{7.39}$ to mutation, changing the human to a rodent version, led to the formation of a polar interaction network now favoring the binding of the propanolamine moiety of adrenergic antagonists. Additionally, the high affinity of norfenfluramine for 5-HT_{2B} receptors, which may lead to valvular heart disease (Rothman et al., 2000), was explained by the orientation of a nonconserved methionine residue ($M^{5.39}$) that was oriented in the binding pocket in the 5-HT_{2B} receptor and absent in all other 5-HT receptors (Wang et al., 2013). Finally, distinctly different patterns of signaling by ergotamine at 5-HT_{1B} receptors, where it is unbiased, and 5-HT_{2B} receptors, where it shows arrestin bias, led to explication of the structural features responsible for functional selectivity (Wacker et al., 2013). Thus, the apparent arrestin-biased signaling by ergotamine at 5-HT_{2B} receptors is due to a preferential stabilization of inactive states of particular G protein conformational microswitches (which are essential for mediating canonical signaling) and the preferential allowance of conformations responsible for arrestin-dependent signaling (Wacker et al., 2013).

More recent studies have clarified the mechanisms responsible for the differential activation of G protein versus arrestin signaling (Wacker et al., 2017a) at 5-HT_{2A} (Wacker et al., 2017b) and 5-HT_{2B} receptors (Wacker et al., 2017b; McCorvy et al., 2018). In particular, a detailed study of several 5-HT_{2B} receptor structures (Wacker et al., 2017b; McCorvy et al., 2018) disclosed that key interactions with $A^{5.46}$ and $T^{3.37}$ are essential for canonical G protein signaling, whereas interactions with extracellular loop residue $L^{7.35}$ are essential for arrestin-ergic signaling. Additionally, other structures of an intermediate-active state of the 5-HT_{2C} receptor (Peng et al., 2018; Fig. 28) and the G protein heterotrimer-stabilized active state of the

5-HT_{1B} receptor (Garcia-Nafria et al., 2018) implicated a key "trigger motif" P-I-F as being essential for the active-inactive switch and biased signaling (Wacker et al., 2017a; McCorvy et al., 2018).

B. 5-HT Ligand-Gated Ion Channels

5-HT_3 receptors are ion channels of the Cys-loop receptor family (Thompson et al., 2010; Corringer et al., 2012). Thus, they share a common architecture with other members, such as nicotinic acetylcholine, ionotropic γ-aminobutyric, and glycine receptors. All of them are composed of five subunits, symmetrically disposed around a central ionic pore axis, forming an urn-like architecture (Fig. 29). Each subunit has three functional domains (Fig. 29): a large extracellular domain harboring the neurotransmitter binding pocket, a four-helix transmembrane domain establishing the ion channel, and an intracellular domain that mediates the receptor interaction with intracellular proteins.

Recent years have seen the emergence of a solid structural framework to help interpret functional and pharmacological studies. First, 5-HT, granisetron, and palonosetron have been crystallized in complex with a soluble model protein (termed 5-HTBP; Kesters et al., 2013; Price et al., 2016), providing possible orientations of ligands and adding up to the huge variety of nicotinic receptor ligands cocrystallized with the same type of model protein. Second, the initial mouse 5-HT_3A receptor X-ray structure (Hassaïne et al., 2014) has provided an almost complete picture (~60 unstructured residues missing in the intracellular domain) of a 5-HT_3 receptor but with empty neurotransmitter sites capped by stabilizing llama antibodies instrumental to crystallization. Third, a series of distinct conformations of the full-length murine receptor imaged by Cryo-Electron Microscopy (Basak et al., 2018a,b; Polovinkin et al., 2018) has shed light on its gating mechanism and revealed how 5-HT and antiemetic drugs such as tropisetron bind in the neurotransmitter site. Specialized reviews discuss Cys-loop receptor structure at length (daCosta and Baenziger, 2013; Nys et al., 2013; Sauguet et al., 2015; Wu et al., 2015; Nemecz et al., 2016).

The five equivalent neurotransmitter binding pockets of the homopentameric 5-HT_3A receptor are located in electronegative clefts at interfaces between two adjacent subunits (Fig. 30). Three loops of the principal subunit and four portions of beta strands of the complementary subunit contribute to the pocket (Fig. 30). Therefore, the shape of the binding pocket arises from the combination of the quaternary arrangement (how one subunit is oriented relative to its neighbor) and of the local conformation of loops and side chains. During activation of Cys-loop receptors and gating of the channel, both global subunit/subunit orientation and more local conformation changes take place (Sauguet et al., 2013; Du et al., 2015; Basak et al., 2018a; Polovinkin et al., 2018). The 5-HTBP and other model proteins present a stiff quaternary structure in which the subunit/subunit orientation is fixed, and in that respect, they are imperfect models for ligand binding.

The binding cleft of the 5-HT_3A receptor is surrounded by aromatic residues forming a 10-Å wide box, including W156 (loop B), W63 (loop D), Y126 (loop E), F199, and Y207 (loop C). W156 (W145 in 5-HTBP) lies at the bottom of the cleft and has cation-Pi interaction with 5-HT

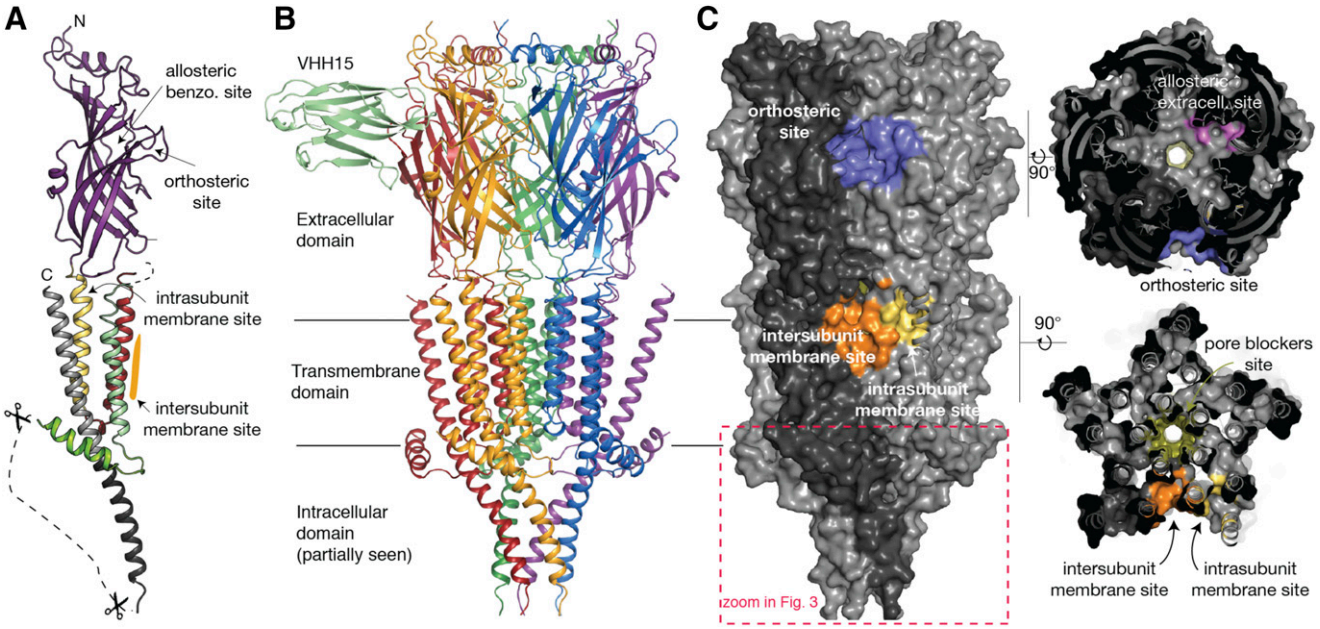

Fig. 29. Architecture and ligand binding sites of 5-HT_3 receptors. (A) Cartoon representation of a single subunit viewed parallel to the plane of the membrane. (B) Cartoon representation of the entire pentameric receptor, in the same orientation [the subunit of (A) is equivalent to the yellow subunit]. One out of the five stabilizing VHH15 single-chain llama antibodies is shown in pale green and labeled VHH15. (C) Surface representation of the receptor highlighting binding clefts in blue (neurotransmitter site), yellow (anesthetics intrasubunit site), orange (PU-02 and anesthetics intersubunit site), purple (extracellular allosteric pocket), and olive (pore blockers site). The subunit equivalent to the one of (A) appears in darker gray.

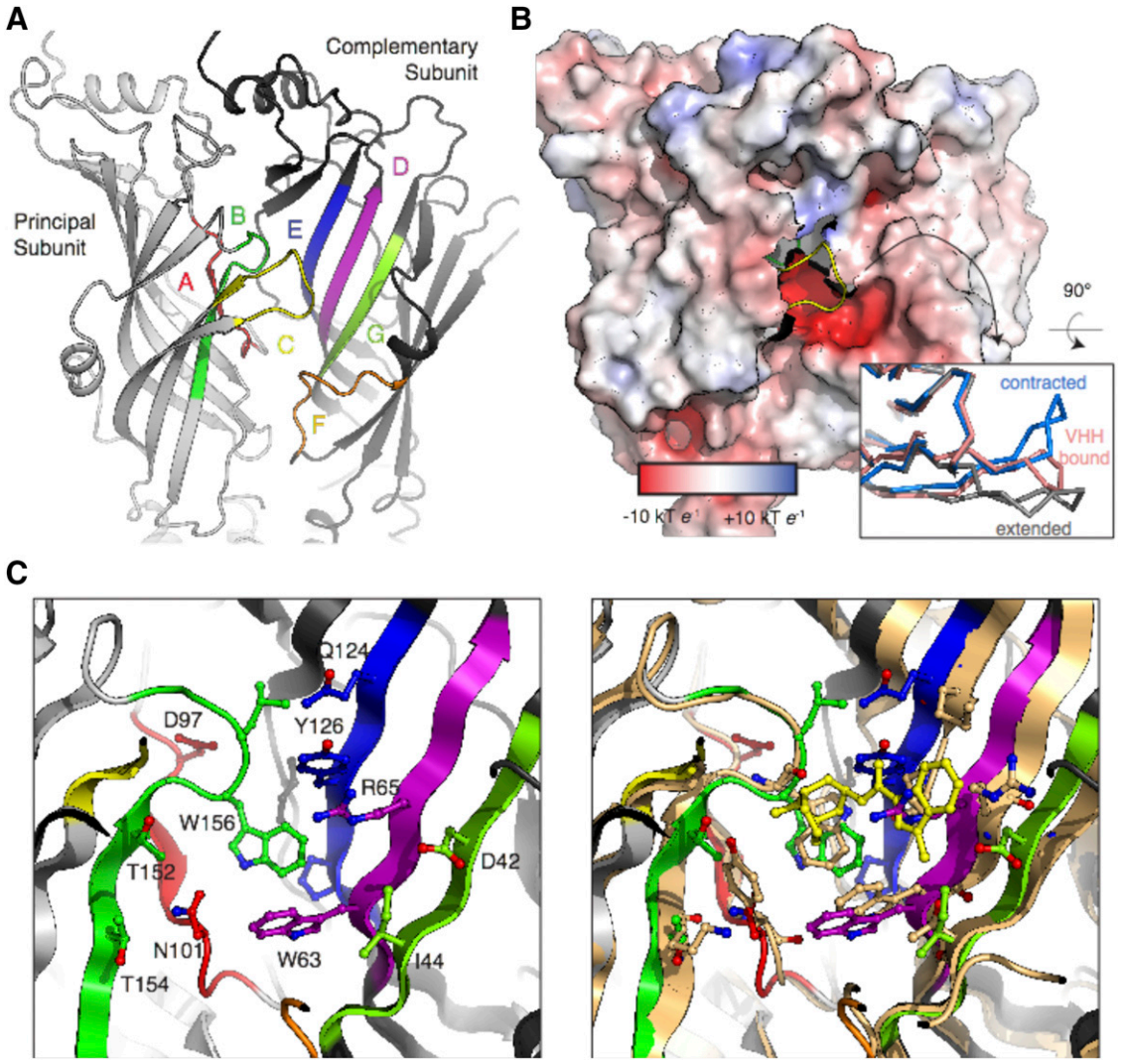

Fig. 30. The neurotransmitter binding site of the 5-HT_3 receptor. (A) Global view of the site, at the interface between two subunits represented as cartoons, viewed from parallel to the place of the membrane. Binding elements of the principal subunit (A–C) and of the complementary subunit (D–G) are color-coded. (B) Surface view representing the electrostatic potential in the same orientation. The surface has been removed around to yellow loop (C) that would cover it, for clarity. The inset illustrates motions of loop (C) associated with binding of an agonist (blue, contracted conformation, PDB 2BYQ), an antagonist (gray, extended conformation, PDB 2C9T), or the stabilizing llama antibody VHH15 (salmon). (C) Close-up views of the binding site with essential residues, including those of the aromatic box. On the right panel, the 5-HT_3A receptor structure is superimposed with the 5-HTBP structure cocrystallized with granisetron (in yellow). The principal subunits are superimposed, and, as a consequence of different subunit/subunit orientation, the strands of the complementary subunit are shifted. This superimposition illustrates the diversity and complementarity of structural templates.

(Beene et al., 2002). This part of the site looks rigid with many intra- and intersubunit interactions. In contrast, the mouth of the cleft seems prone to reorganization. The C loop adopts different conformations in the apo, the antagonist-bound, and 5-HT-bound (Fig. 30). Other important residues lining the site include N101 (loop A), T152 and T154 (loop B), R65 (loop D), D177, S179, I180 (loop F), D42, and I44 (loop G).

A picture of the molecular mechanism of 5-HT_3 receptors starts to emerge from the accumulated structural data. The closed apo conformation resembles those of the receptor inhibited by small molecules (antiemetics such as tropisetron) or by the inhibitory VHH15. Upon 5-HT binding, the receptors undergo a transition to the open state characterized by 1) a compaction of the neurotransmitter pocket because of quaternary reorganization of the extracellular domain and 2) an opening of the transmembrane pore linked to the pivot of each subunit transmembrane domain. Two independent cryo-EM studies have captured a second 5-HT–bound state featuring a closed pore similar to that of the resting state (Basak et al., 2018a; Polovinkin et al., 2018). Based on functional experiments, this state was tentatively described as preactive rather than desensitized (Polovinkin et al., 2018).

There is no direct structural data on allosteric modulation sites of 5-HT_3 receptors. Nevertheless, allosteric sites identified on homologous Cys-loop receptors are relevant to the 5-HT_3 pharmacology. For instance, PU-02, a selective inhibitor of the 5-HT_3 receptor acting in the micromolar range, binds to a transmembrane intersubunit site (orange in Fig. 29)

that correlates to the ivermectin site of the *C. elegans* glutamate-gated chloride channels receptor (Hibbs and Gouaux, 2011) and to the ethanol and bromoform sites of the model bacterial GLIC receptor (Sauguet et al., 2013). In the 5-HT_3A receptor structure, a deep cleft between subunits is indeed accessible from the lipid bilayer and lined by residues determining PU-02 activity [16]: L266, S270, G282, and V288 on the (+) side and S226, I268, and T272 on the (−) side (Fig. 29). A second membrane-accessible site, this time located within a single subunit (Fig. 29), has been identified as the site of action of the positive allosteric modulator TMPPAA (Gasiorek et al., 2016; Polovinkin et al., 2018) and might be involved in anesthetics inhibition (Lopreato et al., 2003). Some compounds acting as physical blockers of the ion flux bind directly in the channel lumen. A variety of sites have been identified at different depths in the pore, spanning almost the whole transmembrane part, from divalent cations binding close to the intracellular mouth to TEA and lidocaine in the middle (Hilf et al., 2010) and mementine that binds close to the extracellular mouth (between 13' and 16') (Rammes et al., 2001; Ulens et al., 2014).

The 5-HT_3 receptor structures also shed light on the neglected intracellular domain. Only part of it is seen in the structures: a short helical stretch after the M3 transmembrane helix and a long helix MA continuous with the M4 transmembrane helix (Figs. 29 and 31). At their N-terminal side, MA helices form a tight pentameric bundle stabilized by hydrophobic interactions (Fig. 31). Further up, and closer to the mouth of the transmembrane pore, they carry charged residues whose mutations were shown to profoundly affect the channel conductance. At this level, the spacing between MA helices results in lateral fenestrations, plugged in the closed-pore structures by the loop linking M3 and MA (Fig. 31). Recent cryo-EM structures have shown that this intracellular region is drastically reorganized in the open-pore state, with wide portals, and displays flexibility. The ion exit pathway and the way in which the ion flux depends on the flexibility and the local electrostatic environment remain to be fully described (Di Maio et al., 2015).

Molecular dynamic simulations established the existence of a dewetted zone in the transmembrane pore in the closed-pore conformations and its conversion to a fully hydrated pore permeant to cations in the open conformations (Yuan et al., 2016; Polovinkin et al., 2018).

XVII. 5-HT GPCRs and Their Interacting Proteins

A. Introduction

The history of cell signaling started more than 60 years ago with the seminal discovery of cAMP by Earl Sutherland (Nobel prize in 1971). The mechanisms by which some receptors activate the production of second messengers has been revealed by Martin Rodbell (Nobel Prize 1994) in the 1970s when he showed that

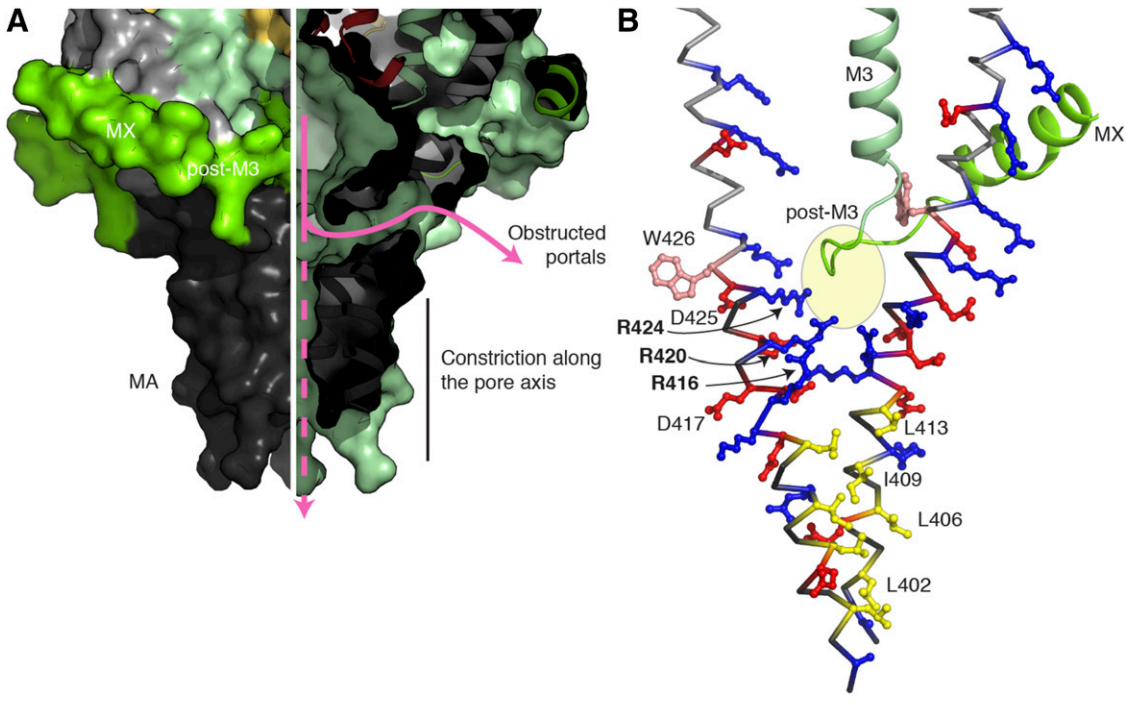

Fig. 31. The intracellular domain of the 5-HT_3 receptor. (A) Surface representation of the intracellular domain viewed parallel to the membrane plane. The left part shows the external surface, whereas the right part shows a cut-through and thus depicts the intracellular vestibule, the lateral obstructed portal, and the constriction along the pore axis. The arrow indicates the plausible exit for ions. (B) Backbone representation of MA-M4 helices (gray, two subunits shown) with the numerous charged residues (blue and red for positively and negatively charged residues) depicted as sticks. The green cartoon shows for one subunit the M3 and MX helices and their connecting loop that plug the portal (yellow oval). The hydrophobic residues that create the tight bundle of MA helices are in yellow, and the triplet of arginine determinant for channel conductance are labeled in bold font.

the receptors do not directly stimulate second messenger synthetizing enzymes but indirectly, via an allosteric activation of G proteins. The GPCR ternary complex consisting of a receptor, a G protein, and an effector, enzyme, or channel was born. Surprisingly, five decades later, the nature and functions of the huge protein complexes comprising GPCRs and GPCR-interacting proteins (GIPs) still remain poorly characterized. During their cellular journey, GPCRs are assisted by GIPs for their proper folding, targeting proper subcellular compartments, trafficking to and out of the plasma membrane, and select several alternative signaling pathways, including G protein–independent pathways. In addition, GIPs are able to allosterically modify the pharmacology of associated GPCR and even activate some GPCRs in the absence of ligands. This section of the review is dedicated to GIPs of 5-HT receptors, which are among the GPCRs for which those proteins are the most extensively characterized, mainly thanks to proteomics screens. Given the large number of 5-HT receptor GIPs identified, it provides a wide overview of their role in GPCR physiology. Recent advances in the role of GIPs in fine-tuning 5-HT_6 receptor signaling and associated physiologic functions, including neurodevelopment and cognition, are particularly highlighted.

The evolution of multicellular organisms has been closely linked to their capacity to communicate with their environment and to develop sophisticated communications between their own cells. Most of these communications involve chemical messengers (e.g., hormones, neurotransmitters, growth factors) that interact with one or several transmembrane receptors. Among those receptors, GPCRs are the most numerous ones (Bockaert and Pin, 1999). Around 1000 genes encode such receptors in the human genome (representing 3% to 4% of the genome), of which ~300 are nonolfactory receptors (Fredriksson et al., 2003). 5-HT receptors are particularly well represented in this important receptor family. The diversity of 5-HT GPCRs is certainly due to molecular tinkering from one ancestral gene and has been instrumental for the implication of 5-HT in a large number of physiologic and pathophysiological functions both in the CNS and peripheral tissues. Because of their structural diversity, 5-HT receptors are able to trigger a large panel of signaling events (Marin et al., 2012). Many of them are transduced by different G proteins. In addition, signaling events elicited by 5-HT receptor activation as well as by many GPCRs are triggered or finely modulated through their interaction with multiple intracellular proteins, "GIPs," and are assembled into functional complexes designated as "receptosomes" (Bockaert et al., 2003, 2004b, 2010b; Maurice et al., 2011; Marin et al., 2012). GIPs are not only involved in GPCR signaling but also in their targeting to subcellular compartments, including axonal and dendritic compartments, in their trafficking in and out of the plasma membrane through the endoplasmic reticulum, Golgi apparatus, endosomal, and lysosomal compartments (Bockaert et al., 2003, 2004b, 2010; Maurice et al., 2011; Marin et al., 2012). Several reviews have been published on GIPs, including two dedicated to 5-HT receptor GIPs (Allen et al., 2008; Marin et al., 2012). Accordingly, only a brief description of the nature, cellular, and physiologic functions of 5-HT receptor GIPs will be provided here (see Table 21), focusing on intracellular GIPs and not considering 5-HT receptor association with other GPCRs (homodimer/heteromer formation). A more detailed account of proteins interacting with the 5-HT_6 receptor is presented to highlight the interest of a comprehensive description of GIPs for a given receptor to unravel novel mechanisms underlying GPCR activation and physiologic functions controlled by those receptors.

B. A Survey of 5-HT Receptor GIPs

1. 5-HT_{1A} Receptor. The 5-HT_{1A} receptor recruits and activates both G_{i3} and G_o (Bockaert et al., 2006; Marin et al., 2012). Coupling to G_{i3} is predominant in the soma of 5-HT raphe neurons and mainly leads to adenylyl cyclase (AC) inhibition, whereas coupling to G_o prevails in hippocampus and leads to inhibition of GIRK channels (Mannoury la Cour et al., 2006); see also Marin et al. (2012) for a detailed review of G_i/G_o-mediated signaling events elicited by 5-HT_{1A} receptor and their physiologic impact. The molecular basis of the difference in G protein coupling is unknown. Only two GIPs are known to interact with 5-HT_{1A} receptors, namely calmodulin (Della Rocca et al., 1999; Turner et al., 2004) and Yif1B (Carrel et al., 2008; Al Awabdh et al., 2012; Alterio et al., 2015). The Ca^{2+}-calmodulin complex binds to two distinct sites located in the third intracellular loop (i_3) of the receptor (Turner et al., 2004), whereas Yif1B (Yip1 interacting factor homolog B) associates with its short C-terminal domain (Ct). Ca^{2+}-calmodulin is implicated in receptor internalization, Erk1/2 activation, and modulation of PKC-dependent receptor phosphorylation (Della Rocca et al., 1999; Turner et al., 2004). In addition, Ca^{2+}-calmodulin contributes to receptor-operated Janus kinase 2 (Jak2) and type 1 sodium-proton exchanger (NHE-1) activation (Turner et al., 2007). 5-HT_{1A} receptor activation leads to the assembly of a ternary signaling complex that includes activated Jak2, Ca^{2+}-calmodulin, and NHE-1, in which calmodulin is activated by tyrosine phosphorylation instead of cellular Ca^{2+} elevation. This results in tighter interaction of calmodulin with NHE-1, NHE-1 conformational change, and increased NHE-1 transport activity (Turner et al., 2007). Yif1B is a protein implicated in the traffic of proteins from the endoplasmic reticulum (ER) to the Golgi (Alterio et al., 2015) that plays a key role in the selective targeting of 5-HT_{1A} receptors to dendrites (Carrel et al., 2008). It has been proposed that Yif1B serves as a scaffold allowing the recruitment of 5-HT_{1A} receptor to a complex comprising

TABLE 21
5-HT receptor–interacting proteins and their cellular and physiologic functions

For each receptor, the nature of identified GIPs, their site of interaction, the function of their interaction with the receptor (if known) at the cellular (receptor targeting, trafficking, and signal transduction) and physiologic levels as well as their role in pathologies are indicated. The question marks indicate that the site of interaction of the GIP in the receptor sequence or the physiologic/pathologic functions of its interaction with the receptor remain unknown.

Receptor	GIP	Site of Interaction	Cellular Functions	Physiologic/Pathologic Functions	References
5-HT_{1A}	Calmodulin	i_3/Ct	Endocytosis PKC-dependent receptor phosphorylation Erk1,2 and Jun kinase activation NHE-1 activation	?	Della Rocca et al., 1999; Turner et al., 2004, 2007
	Yif1B	Ct	Anterograde trafficking Dendritic targeting	?	Al Awabdh et al., 2012; Alterio et al., 2015
5-$HT_{1B/D}$	p11	i_3	↗Cell surface density and signaling	↘Depression in rodent models ↘Emotional memory in rodents	Svenningsson et al., 2006; Eriksson et al., 2013
5-HT_{2A}	β-arrestin 2	?	↗PI3K/Src/Akt signaling Delayed recycling (human receptor)	Hallucinogenic effects induced by high 5-HT concentration	Schmid et al., 2008; Bhattacharya et al., 2010; Schmid and Bohn, 2010
	MAP1A	?	Trafficking in apical dendrites		Allen et al., 2008
	Arf1-6	i_3/Ct	Coupling to phospholipase D		Robertson et al., 2003
	Jak/STAT3	?	↗Transcription		Guillet-Deniau et al., 1997
	RSK2	i3	Receptor phosphorylation and desensitization	Putative implication in psychiatric disorders (Coffin-Lowry syndrome)	Sheffler et al., 2006; Allen et al., 2008; Strachan et al., 2009
5-HT_{2A}	Calmodulin	i_2/Ct	↘Gq coupling		Turner and Raymond, 2005
	Caveolins	?	Targeting to lipid rafts Endocytosis		Bhatnagar et al., 2004
	PSD-95	Ct (PDZ ligand-SCV)	Dendritic targeting Postsynaptic localization ↗Phospholipase C ↗Endocytosis Cell surface density	Hallucinogen effects in mice Antipsychotic drug effects ↘Inflammatory and neuropathic pain	Xia et al., 2003; Becamel et al., 2004; Abbas et al., 2009; Pichon et al., 2010; Vogrig et al., 2013; Wattiez et al., 2013
	SAP97	Ct (PDZ ligand-SCV)	↗Gq signaling ↘Endocytosis	?	Allen et al., 2008
	MUPP1	Ct (PDZ ligand-SCV)	Postsynaptic localization ↗Cell surface density	Drug dependence	Jones et al., 200; Becamel et al., 2001; Shirley et al., 2004
	MAGI2	Ct (PDZ ligand-SCV)	Postsynaptic localization	?	Bécamel et al., 2004
	CIPP	Ct (PDZ ligand-SCV)	Postsynaptic localization	?	Bécamel et al., 2004
	NHERF3	?	↗Gq signaling ↘Endocytosis	?	Walther et al., 2015
5-HT_{2B}	MUPP1	Ct (PDZ ligand-SYV/I)	?	?	Becamel et al., 2001
	PDZ protein	Ct (PDZ ligand-SYV/I)	NO synthesis	?	Manivet et al., 2000
5-HT_{2C}	Calmodulin	Ct	↗Erk1/2 signaling	?	Bécamel et al., 2002; Labasque et al., 2008
	β-arrestins	i_2/?	↗Erk1/2 signaling Constitutive and agonist-dependent endocytosis	?	Labasque et al., 2008, 2010; Marion et al., 2004
	MUPP1	Ct (PDZ ligand-SSV)	?	?	Becamel et al., 2001; Parker et al., 2003
	PSD-95	Ct (PDZ ligand-SSV)	↗Endocytosis ↘Signaling	?	Bécamel et al., 2002; Gavarini et al., 2006
	MPP3	Ct (PDZ ligand-SSV)	↘Endocytosis	?	Bécamel et al., 2002; Gavarini et al., 2006
	Veli3/CASK/MintMAGI2, SAP102, PSD93	Ct (PDZ ligand-SSV)	?	?	Bécamel et al., 2002
5-HT_{2C}	PICOT	Ct	?	?	Bécamel et al., 2002
	PTEN	i_3	?	Rewarding effects of THC and nicotine	Ji et al., 2006
5-HT_4	Src	?	↗Erk1/2 signaling ↗PLCγ1	?	Gill et al., 2005; Barthet et al., 2007; Barthet et al., 2009
	p11	i_3	↗Cell surface localization ↗Signaling	↘Depression in rodent models	Warner-Schmidt et al., 2009
	ADAM10	?	↗sAPPα release	↘β-amyloid plaques ↘Neuroinflammation	Cochet et al., 2013
	GRK5	Ct	↘Src activation ↘Erk1/2 signaling	?	Barthet et al., 2009
5-$HT_{4(a)}$	SNX27a		Targeting to endosomes	?	Joubert et al., 2004

(*continued*)

TABLE 21—*Continued*

Receptor	GIP	Site of Interaction	Cellular Functions	Physiologic/Pathologic Functions	References
		Ct (PDZ ligand-SCF)	Receptor recycling?		
	NHERF	Ct (PDZ ligand-SCF)	Targeting to microvilli - interaction with Ezrin	?	Joubert et al., 2004
	CRMP2	Ct	Neuronal architecture	?	Joubert et al., 2004
5-$HT_{4(b)}$	PDE3A1, PDE4D3	?	Regulation of cAMP signaling	?	Weninger et al., 2014
5-$HT_{4(e)}$	CIPP	Ct (PDZ ligand-VPV)	?	?	Joubert et al., 2004
5-$HT_{4(e)}$	nNOS	Ct (PDZ ligand-VPV)	?	?	Joubert et al., 2004
5-HT_6	Fyn	Ct (PXHPXR)	↗Gs signaling ↗Erk1/2 signaling ↗Cell surface expression	?	Yun et al., 2007
	Jab1	i_3/Ct	↗Gs signaling ↗Cell surface expression ↗c-Jun phosphorylation	↘Cell death	Yun et al., 2010
	Map1B-LC1	Ct	↗Cell surface expression ↘Endocytosis ↗Erk1/2 signaling	?	Kim et al., 2014
	SNX14	i_3	↗Endocytosis ↘Gs signaling	?	Ha et al., 2015
	mTORC1	Ct/unknown	↗mTOR signaling	Cognitive impairment in neurodevelopmental models of schizophrenia	Meffre et al., 2012
	Cdk5, p35	Ct	Receptor phosphorylation Cdk5 signaling	Neurite growth migration of pyramidal neurons	Duhr et al., 2014; Jacobshagen et al., 2014; Dayer et al., 2015
5-HT_7	Neurochondrin		?	?	Ward et al., 2009
	Periplakin		?	?	Ward et al., 2009
	S100B	i_3	↘cAMP	Behavioral despair	Stroth and Svenningsson, 2015

PICO, protein kinase C interacting cousin of thioredoxin.

Rab6, kinesin family member 5B, and dynein that coordinates its anterograde traffic to terminal dendrites (Al Awabdh et al., 2012).

2. 5-HT_{1B} Receptor. In addition to its coupling to G_i/G_o (Bockaert et al., 2006; Marin et al., 2012), the 5-HT_{1B} receptor interacts via its i_3 loop with a particularly interesting GIP, named p11 (Svenningsson et al., 2006; Svenningsson and Greengard, 2007). p11 (also known as S100A10) is a member of the S100 EF-hand protein family. S100 proteins are small acidic proteins (10–12 kDa) characterized by two calcium-binding sites that have helix-loop-helix ("EF-hand type"). p11 increases cell surface density of 5-HT_{1B} receptor and, thus, receptor-operated signaling (Svenningsson et al., 2006). Reduction in p11 level has been found in postmortem human tissue from depressed individuals and suicide victims as well as in a rodent model of depression, whereas its level in rodent brain is increased by antidepressants or electroconvulsive therapy (Svenningsson et al., 2006; Svenningsson and Greengard, 2007). Furthermore, invalidation of the gene encoding p11 induces a depression-like phenotype, reduces response to antidepressants and emotional memory, and enhances dependence to cocaine (Svenningsson and Greengard, 2007; Eriksson et al., 2013; Svenningsson et al., 2013; Svenningsson, 2014).

3. 5-HT_{2A} Receptor. The 5-HT_{2A} receptor is mainly coupled to G_q/G_{11} proteins. 5-HT_{2A} receptors also engage G_i/G_o-mediated signaling upon stimulation by hallucinogenic agonists, including LSD and synthetic agonists such as DOI (Bockaert et al., 2006; Gonzalez-Maeso et al., 2007; Marin et al., 2012; Karaki et al., 2014). Like many GPCRs, the 5-HT_{2A} receptor also recruits β-arrestins following agonist stimulation (Bhattacharya et al., 2010). Intriguingly, it has been found that β-arrestin 2 is specifically involved in the hallucinogenic-like effects elicited by high concentrations of 5-HT (or its metabolites) but not in those induced by DOI (Schmid et al., 2008). These hallucinogenic-like effects are mediated by the activation, via β-arrestin 2, of a PI3K/Src/Akt signaling cascade (Schmid and Bohn, 2010). Likewise, β-arrestins are required for Erk1/2 activation by 5-HT but not by DOI (Schmid et al., 2008).

The 5-HT_{2A} receptor (like several other 5-HT receptors, including 5-HT_{2B} and 5-HT_{2C} receptors; see below) expresses a canonical recognition motif for PDZ domain–containing proteins at its C-terminal extremity. PDZ domain–containing proteins (or PDZ proteins) are scaffolding proteins containing one or several PDZ domains, in addition to other protein-protein interaction domains. PDZ domains have been classified according to their specificity for PDZ motifs (or PDZ ligands):

class I PDZ domains preferentially bind to -S/Txφ, class II to -φxφ, and class III to -E/Dxφ motifs (where φ represents a hydrophobic residue and X any residue), respectively. The 5-HT_{2A} receptor PDZ ligand (-SCV) clearly belongs to class I. Several PDZ proteins have been found to interact with the 5-HT_{2A} receptor. The majority of them have been identified thanks to affinity purification coupled to mass spectrometry (AP-MS) proteomic strategies or two-hybrid screens. These include Multi-PDZ protein1 (MUPP1), PSD-95, membrane-associated guanylate kinase with inverted domain structure2 (MAGI2), synapse-associated protein (SAP)97, channel interacting PDZ protein (CIPP), and MAGUK p55 subfamily member 2 (MPP2) and 3 (MPP3) (Becamel et al., 2001; Bécamel et al., 2004; Pichon et al., 2010). Among the 5-HT_{2A} receptor GIPs identified, PSD-95 is certainly the most extensively studied with respect to the functional impact of its association with the receptor. PSD-95, which is located in postsynaptic spine membranes together with other receptor PDZ partners, is required for dendritic targeting of the receptor (Xia et al., 2003; Allen et al., 2008). In addition, microtubule-associated protein (MAP)1A may scaffold the receptor to the microtubule cytoskeleton and facilitate its trafficking to apical dendrites (Allen et al., 2008). PSD-95, like SAP97 and MUPP1, increases receptor cell surface density likely by inhibiting receptor endocytosis (Allen et al., 2008; Jones et al., 2009). The impact of the 5-HT_{2A} receptor/PSD-95 complex upon receptor-operated signaling is less clear. On the one hand, 5-HT_{2A} receptor–mediated downstream signaling is impaired in PSD-95–deficient mice (Abbas et al., 2009), suggesting that the interaction promotes receptor-operated signal transduction. On the other hand, acute disruption of 5-HT_{2A} receptor/PSD-95 association with an interfering peptide increases receptor-mediated cytosolic Ca^{2+} increase and its antihyperalgesic effects in models of neuropathic and inflammatory pain in the rat (Pichon et al., 2010; Wattiez et al., 2013), suggesting that this interaction inhibits receptor-operated signaling. These opposite effects of sustained (PSD-95 knockout) and acute (interfering peptide) disruption of the 5-HT_{2A} receptor/PSD-95 interaction may reflect long-term adaptive changes in PSD-95 knockout mice. In line with the antihyperalgesic effects produced by disruption of 5-HT_{2A} receptor/PSD-95 interaction with a peptide, docking simulation studies identified a series of substituted indoles as potential small-molecule inhibitors of this interaction. One of them efficiently inhibited mechanical hyperalgesia in an experimental model of traumatic neuropathic pain in the rat (Vogrig et al., 2013). Collectively, these studies identify the 5-HT_{2A} receptor/PSD-95 complex as a new therapeutic target for the treatment of neuropathic pain, which remains poorly controlled by currently available analgesics. Consistent with the inhibition of 5-HT_{2A} receptor–operated signaling in PSD-95 deficient mice, the hallucinogenic-like effects of drugs are impaired in these mice (Abbas et al., 2009). Likewise, deletion of PSD-95 renders ineffective atypical antipsychotics such as clozapine, which act as 5-HT_{2A} receptor inverse agonists, against PCP-induced disruption of prepulse inhibition, a widely used pharmacological model of schizophrenia (Abbas et al., 2009). Na^+/H^+ exchanger regulatory factor (NHERF) 3 is another PDZ protein that interacts with the 5-HT_{2A} receptor but, surprisingly, in a PDZ-independent manner (Walther et al., 2015). NHERF3 negatively regulates 5-HT_{2A} receptor endocytosis and positively influences 5-HT_{2A} receptor–stimulated inositol phosphate formation (Walther et al., 2015).

Several 5-HT_{2A} receptor–interacting proteins have been identified in addition to PDZ proteins. Targeting of 5-HT_{2A} receptor to caveolae microdomains is ensured by its interaction with the multifunctional proteins caveolins (Allen et al., 2008). Moreover, association of the receptor with caveolins has a profound impact on receptor-operated signal transduction and functions; interaction of 5-HT_{2A} receptor with caveolin-1 within lipid raft/caveolae membranes enhances receptor-mediated signal transduction by facilitating its coupling to $G\alpha_q$ protein in C6 glioma cells (Bhatnagar et al., 2004), whereas its interaction with caveolin-3, the predominant caveolin isoform in heart, upon 5-HT stimulation, negatively regulates receptor-mediated hypertrophy in cardiomyoblasts and neonatal cardiomyocytes (Mialet-Perez et al., 2012).

ARF1 and, to a lesser extent, ARF6, associate with the i_3 loop and Ct of the 5-HT_{2A} receptor (Robertson et al., 2003). This association is enhanced by GTP loading and is essential for 5-HT_{2A} receptor–mediated PLD activation, which seems to be independent of $G_{q/11}$ (Robertson et al., 2003). 5-HT_{2A} receptors also bind to the tyrosine kinase Jak2 in fetal myoblasts (Guillet-Deniau et al., 1997). This is accompanied by Jak2 autophosphorylation, the recruitment of the transcription factor signal transducer and activator of transcription (STAT)3, and its translocation to the nucleus (Guillet-Deniau et al., 1997). P90 ribosomal S6 kinase 2 (RSK2) interacts with 5-HT_{2A} receptor i_3 loop and exerts a tonic break on receptor-operated signaling (Sheffler et al., 2006; Allen et al., 2008). RSK2 phosphorylates 5-HT_{2A} receptor at Ser^{314} located in the i_3 loop. Ser^{314} phosphorylation underlies heterologous desensitization of the receptor elicited by growth factors (Allen et al., 2008; Strachan et al., 2009). A role of 5-HT_{2A} receptors in symptoms associated with Coffin-Lowry syndrome (because of null mutations in *RSK2* gene), such as moderate to severe mental retardation, movement disorders, and schizophrenia-like psychosis, in heterozygote females has been proposed (Allen et al., 2008). Calmodulin interacts in a Ca^{2+}-dependent manner with consensus binding motifs located in 5-HT_{2A} receptor i_2 loop and Ct. This association inhibits receptor coupling to G_q (Turner and Raymond, 2005).

4. 5-HT_{2B} Receptor. The 5-HT_{2B} receptor is mainly coupled to G_q [see also Bockaert et al. (2006), Marin

et al. (2012) for detailed review of G_i/G_o-mediated signaling events elicited by this receptor].

A number of recognized signaling proteins interact with the 5-HT_{2B} receptor, including constitutive and inducible NOS and the G proteins Gαq, Gα11, and Gα13. The 5-HT_{2B} receptor also binds to the multivalent PDZ scaffold protein, MUPP1; this is in common with the other 5-HT_2 receptors 5-HT_{2A} and 5-HT_{2C}. 5-HT_{2A} and 5-HT_{2B} receptors also bind MUPP1-PDZ domains in vitro and share the C-terminal -E-X-V/I-S-X-V sequence (Becamel et al., 2001). This PDZ motif is also required for the recruitment of the constitutive NO synthase cNOS (Manivet et al., 2000).

Ubiquitin E3 ligases (E3s) confer specificity to ubiquitination by recognizing target substrates. The ligand of numb protein X (LNX) family of E3s is a group of PDZ domain–containing RING-type E3 ubiquitin ligases. The substrate recognition mechanism of LNX E3s involves their specific PDZ domains by binding to the C termini of the target proteins. The human C-terminal LNX1 PDZ3-binding motifs of the 5-HT_{2B} receptor promotes ubiquitination by LNX1ΔPDZ4 (Guo et al., 2012).

5. 5-HT_{2C} Receptor. The 5-HT_{2C} receptor activates phospholipase C (PLC) via $G_{q/11}$, PLD via G_{13}, phospholipase A2 (PLA2), and Akt/glycogen synthase kinase (GSK)3β via Gi_3 (Bockaert et al., 2006). 5-HT_{2C} receptors also activate Erk1/2 signaling via a mechanism entirely independent of G proteins but dependent of their interaction with both Ca^{2+}/calmodulin and β-arrestin 2 (Labasque et al., 2008; Marin et al., 2012). As already mentioned, the receptor expresses a canonical class I PDZ ligand at its C-terminal extremity. MUPP1 was the first PDZ protein identified as a partner of the 5-HT_{2C} receptor in 1998, using the yeast two hybrid system (Ullmer et al., 1998). MUPP1 contains 13 PDZ domains (it is the PDZ protein that contains the largest number of PDZ domains identified) and selectively interacts with the receptor via its 10th PDZ domain (Becamel et al., 2001). This interaction is dynamically regulated by agonist-dependent receptor phosphorylation at Ser^{458} located in the PDZ binding motif (Parker et al., 2003). The gene encoding MUPP1 (*Mpdz*) has been identified as a quantitative trait underlying physical dependence to drugs of abuse (Shirley et al., 2004). Additional PDZ partners of 5-HT_{2C} receptor have been identified by means of AP-MS proteomic strategies (Bécamel et al., 2002, 2004). These include PSD-95, SAP102, MPP3, and the ternary complex Veli3/CASK/Mint1. These studies revealed that in spite of their similar PDZ binding motifs, 5-HT_{2A} and 5-HT_{2C} receptors bind to different sets of PDZ proteins that differ in their synaptic localizations: 5-HT_{2A} receptors preferentially interact with PDZ proteins exclusively located at the postsynapse (e.g., PSD-95, SAP97, and CIPP), whereas 5-HT_{2C} receptors associate with PDZ proteins exhibiting both presynaptic and postsynaptic localizations such as the ternary complex Veli3/CASK/Mint1, consistent with their different distribution at the synapse (Bécamel et al., 2004). 5-HT_{2C} receptor PDZ partners differentially regulate receptor endocytosis and desensitization: PSD-95 increases both constitutive and agonist-induced receptor endocytosis and desensitization, whereas MPP3 stabilizes the receptor at the plasma membrane and prevents its desensitization (Gavarini et al., 2006). Note that PSD-95 has an opposite effect on 5-HT_{2A} and 5-HT_{2C} receptor endocytosis (Xia et al., 2003; Gavarini et al., 2006).

5-HT_{2C} receptors also interact via their i_3 loop with PTEN, a phosphatase that negatively controls the Akt/PkB signaling pathway and thus is a tumor suppressor (Ji et al., 2006). The 5-HT_{2C} receptor/PTEN interaction was established in the VTA, the site of inhibition of the rewarding effects of many drugs of abuse by 5-HT_{2C} receptor agonists. This inhibition is linked to the ability of receptors to prevent the increase in the firing rate of VTA dopaminergic neurons projecting to the nucleus accumbens elicited by drugs. Disrupting the 5-HT_{2C} receptor-PTEN interaction with a cell-penetrating interfering peptide reduced the firing rate of VTA dopaminergic neurons and prevented rewarding effects of drugs of abuse such as THC and nicotine, suggesting that this interaction might be a relevant target for the treatment of drug addiction (Ji et al., 2006). 5-HT_{2C} receptors also interact with protein kinase C interacting cousin of thioredoxin, a PKC theta-interacting protein with a thioredoxin homology domain involved in the regulation of the thioredoxin system (Bécamel et al., 2002). The functional consequence of this interaction remains to be established.

6. 5-HT_4 Receptors. 5-HT_4 receptors engage signaling pathways through multiple G proteins, especially G_S and in some cell lines G_{13}, G_q, and G_i [for reviews, see Coupar et al. (2007), Bockaert et al. (2008)]. In addition, 5-HT_4 receptors stimulate Erk1/2 in a G protein– and β-arrestin–independent mechanism that requires activation of the tyrosine kinase Src constitutively associated with 5-HT_4 receptor (Gill et al., 2005; Barthet et al., 2007; Bockaert et al., 2011). This pathway is also implicated in the activation of PLCγ1-mediated inhibition of NHE-1 in intestinal epithelial cells (Gill et al., 2005). Furthermore, GRK5 interacts with the proximal Ct of 5-HT_4 receptors and phosphorylates β-arrestin 1. This prevents Src activation and underlies desensitization of Src-dependent Erk1/2 activation by 5-HT_4 receptors (Bockaert et al., 2008; Barthet et al., 2009).

Like 5-HT_{1B} receptors, 5-HT_4 receptors interact with p11 via their i_3 loop (Warner-Schmidt et al., 2009). p11 enhances receptor cell surface localization and signal transduction (Warner-Schmidt et al., 2009). Given the extensive data showing that p11 is downregulated in depressive states (Svenningsson and Greengard, 2007) and that activation of 5-HT_4 receptors has antidepressant effects (Ge and Barnes, 1996; Bockaert et al., 2011),

this interaction might be of particular interest for the treatment of depression.

The 5-HT_4 receptor interacts, directly or indirectly, with the α-secretase ADAM10. This interaction might be an important step in the constitutive (agonist-independent) activation of nonamyloidogenic cleavage of amyloid precursor protein (APP) and release of soluble APPα (sAPPα) fragment elicited by the receptor (Cochet et al., 2013). sAPPα has neurotrophic and neuroprotective properties, and its release on constitutive (and agonist-dependent) receptor activation might contribute to receptor-mediated reduction of amyloid pathology observed in several mouse models of Alzheimer disease (Cochet et al., 2013; Tesseur et al., 2013; Pimenova et al., 2014; Claeysen et al., 2015).

The 5-HT_4 receptor is one of the GPCRs for which alternative mRNA splicing generates the most variants that differ in their Ct, with some variants (a, e, and f) expressing canonical PDZ ligands, suggesting that these receptors may recruit specific GIPs, including different sets of PDZ partners. Ten proteins have been shown to interact with the Ct of the 5-$HT_{4(a)}$ receptor. Most of them are PDZ proteins that associate with its canonical class I PDZ motif (-SCF) (Joubert et al., 2004). These include SNX27a, a member of the sorting nexin family, which targets 5-$HT_{4(a)}$ receptors to early endosomes in cell lines (Joubert et al., 2004). A more recent study showed that SNX27 associates with β_2-adrenergic receptor PDZ ligand and that this interaction is essential for PDZ-directed receptor recycling (Temkin et al., 2011). It also showed that SNX27 serves as an essential adaptor protein linking PDZ motif containing cargo to the retromer. SNX27 also directly interacts with endosomes through its lipid-binding phox homology (PX) domain. A similar role of SNX27a in endocytic 5-HT_4 receptor trafficking is likely. The interaction of the 5-$HT_{4(a)}$ receptor with the PDZ protein NHERF promotes its recruitment to microvilli, where it localizes with activated ezrin, consistent with a role of 5-HT_4 receptors in cytoskeleton remodeling (Joubert et al., 2004). The Ct of the 5-$HT_{4(a)}$ receptor also recruits CRMP2, a member of the collapsing response mediator protein (CRMP) family through a PDZ-independent mechanism (Joubert et al., 2004). 5-HT_4 receptors cause G_{13}- and RhoA-dependent neurite retraction and cell rounding in neuroblastoma cells (Ponimaskin et al., 2002b). Their association with CRMP2 might also contribute to regulation of neuronal architecture.

Three GIPs associate with the class II PDZ ligand (-VPV) of the 5-$HT_{4(e)}$ receptor. These include two PDZ partners, namely CIPP and nNOS, the only NOS isoform expressing a PDZ domain. The third protein that binds to the Ct of 5-$HT_{4(e)}$ receptor is Sec23A, a protein of the COPII complex, which is likely involved in its trafficking from the ER to the Golgi (Joubert et al., 2004). The 5-$HT_{4(b)}$ receptor interacts with the phosphodiesterases PDE3A1 and PDE4D3, which may thereby regulate receptor-operated cAMP signaling (Weninger et al., 2014).

7. 5-HT_7 Receptor. The 5-HT_7 receptor is coupled to G_s and G_{12} (Bard et al., 1993; Ruat et al., 1993b). 5-HT_7 receptor stimulation promotes neurite outgrowth and SRE-mediated gene transcription through Gα_{12}-dependent activation of RhoA and Cdc42 GTPases (Kvachnina et al., 2005; Guseva et al., 2014b). The 5-HT_7 receptor Ct interacts with neurochondrin, a protein expressed in bone and brain that promotes neurite growth. Neurochondrin interacts with several GPCRs, including melanin-concentrating hormone receptor 1, orexin-1 and thromboxane A2 receptors, and the mGlu5 metabotropic glutamate receptor (Ward et al., 2009; Marin et al., 2012). Generally, neurochondrin inhibits GPCR coupling to G proteins. The 5-HT_7 receptor Ct also recruits the intermediate filament periplakin, which, like neurochondrin, inhibits receptor-operated Ca^{2+} signaling (Ward et al., 2009). The role of neurochondrin in neurite outgrowth elicited by 5-HT_7 receptor stimulation remains unexplored.

5-HT_7 receptors, via their i_3 loop, interact with S100B, a member of the S100 family, and S100B negatively regulates 5-HT_7 receptor–induced cAMP production (Stroth and Svenningsson, 2015). S100B seems to play a role in the antidepressant effects of fluoxetine (Baudry et al., 2010), and its serum concentration may serve as a biomarker predicting response to antidepressant treatment (Arolt et al., 2003). Notably, 5-HT_7 receptor antagonists have been reported to display rapid antidepressant action, making the receptor a promising target for antidepressants with improved onset of clinical efficacy compared with most of the currently available treatments (Mnie-Filali et al., 2011). Suggestive of a possible influence of 5-HT_7 receptor/S100B interaction in the pathogenesis of mood disorders and their treatment, transgenic female mice overexpressing S100B show depressive-like behavior that is normalized by administration of a 5-HT_7 receptor antagonist (Stroth and Svenningsson, 2015).

The human and rat genes coding for the 5-HT_7 receptors generate different splice variants (Heidmann et al., 1997). One of them (5-$HT_{7(b)}$ receptor) is common to rat and human and expresses at its extreme Ct a canonical class II PDZ ligand (-FVL). To date, no PDZ partner of 5-$HT_{7(b)}$ receptor has been identified.

C. 5-HT_6 Receptor Receptosome: Toward New Signaling Mechanisms Underlying Its Control of Cognition and Neurodevelopmental Processes

1. Introduction. The 5-HT_6 receptor has early been considered as a promising target for psychiatric diseases in line with its near exclusive expression in the central nervous system (Hirst et al., 2003). Although initial studies suggested that 5-HT_6 receptors are almost exclusively localized on GABA interneurons, more recent studies, including some in human brain,

indicate that they are also present on pyramidal glutamatergic neurons in prefrontal cortex and hippocampus (Woolley et al., 2004; Marazziti et al., 2013b). A more detailed study of 5-HT_6 receptor mRNA distribution showed highest expression in both D_1 and D_2 receptor–containing medium-size spiny neurons of caudate putamen and nucleus accumbens and confirmed consistent expression in glutamatergic neurons of hippocampus and cerebral cortex expressing vGluT1 (Helboe et al., 2015). It also showed the presence of 5-HT_6 receptor mRNA in a minor fraction of GABAergic cortical neurons, including mainly neurons coexpressing 5-HT_{3A} receptor and a subset of calbindin- and calretinin-positive neurons (Helboe et al., 2015). 5-HT_6 receptors thus display an ideal distribution to regulate the balance between excitatory and inhibitory signaling in brain regions implicated in cognition, which is altered in schizophrenia (Uhlhaas and Singer, 2010). The pharmacology of 5-HT_6 receptors includes a great number of ligands exhibiting agonist or antagonist activities. Some of them have shown putative interest for treating cognitive deficits, depression, anxiety, sleep, and feeding disorders as well as pain (Mitchell and Neumaier, 2005; Svenningsson et al., 2007; Fone, 2008; Ly et al., 2013; Wilkinson et al., 2014; Claeysen et al., 2015; Karila et al., 2015; Pereira et al., 2015). Unfortunately, phase III clinical trial results for treating cognitive impairment have been disappointing. An increase in our understanding of how the cellular and molecular events associated with the 5-HT_6 receptor translate to behavioral responses may inform and support stratified medicine approaches.

The 5-HT_6 receptor is positively coupled to G_s and, like many GPCRs, activates Erk1/2 (Ruat et al., 1993a; Sebben et al., 1994) (Fig. 32). As G_s/cAMP and Erk1/2 pathways can have a positive influence on cognition, it was unlikely that their inhibition would mediate the procognitive effects of 5-HT_6 receptor antagonists. This suggested that alternative coupling mechanisms might be involved. Likewise, the cellular mechanisms controlling 5-HT_6 receptor functional activity remained largely unexplored until the use of unbiased strategies to identify 5-HT_6 receptor–interacting proteins. These included two-hybrid screens (Yun et al., 2007, 2010; Kim et al., 2014) and AP-MS proteomic strategies based on different methods to purify receptor partners, such as coimmunoprecipitation (Meffre et al., 2012) or pull-down assays using particular receptor sequences as baits (Duhr et al., 2014; Ha et al., 2015).

2. Fine-Tuning of 5-HT_6 Receptor Trafficking and Signal Transduction by GIPs. The nonreceptor tyrosine kinase Fyn was the first GIP discovered for 5-HT_6 receptor, thanks to a two-hybrid screen using the receptor Ct as bait (Fig. 32). Fyn is a member of the Src family of tyrosine kinases that are highly expressed in brain and involved in synaptic plasticity. Several lines of evidence suggest that Fyn might be implicated in Alzheimer disease pathogenesis: Fyn phosphorylates Tau at Tyr^{18} and controls its association with microtubules (Lee et al., 2004); it has also been involved in Aβ-induced synaptic deficits and neurotoxicity (Yang et al., 2011). In addition, Fyn plays a key role in neurodevelopmental processes: in combination with Src, Fyn controls cortical lamination, the formation of

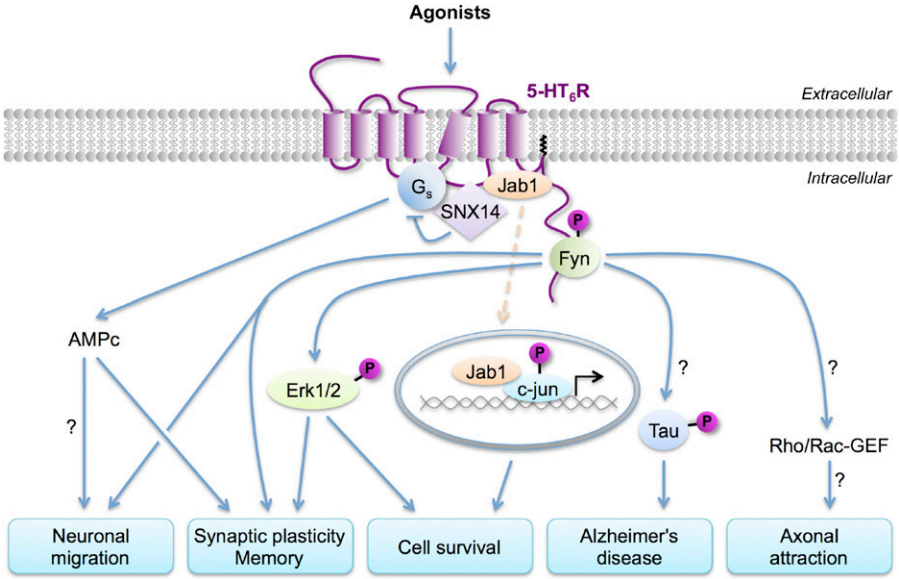

Fig. 32. Role of Fyn, Jab1, and SNX 1 recruitment by 5-HT_6 receptor in receptor-operated signaling and functions. Association of Fyn with 5-HT_6 receptor Ct increases receptor cell surface localization and, consequently, receptor-operated G protein signaling. Fyn is also involved in Erk1/2 activation by 5-HT_6 receptors. Fyn phosphorylates Tau to control its association with microtubules and is involved in neuronal migration and Aβ-induced synaptic deficits and neurotoxicity. Association of 5-HT_6 receptor with Jab1 stabilizes surface expression of the receptor and is essential for its activity. 5-HT_6 receptor stimulation increases nuclear translocation of Jab1, a process that might reduce cell death induced by hypoxia. The 5-HT_6 receptor also interacts with SNX14, which inhibits receptor functions by sequestering $G\alpha_s$ and promoting receptor endocytosis and degradation.

the Purkinje cell plate, and, thus, neuronal migration (Kuo et al., 2005). It is involved in netrin-1–mediated axon attraction of cortical neurons via the phosphorylation of Trio, a Rho/Rac-GEF (DeGeer et al., 2013), and in the maturation of GABAergic synapses elicited by neural cell adhesion molecule (Chattopadhyaya et al., 2013). Fyn binds to 5-HT_6 receptor Ct (likely to the conserved polyproline sequence PXhPXR, where X represents any amino acid and h any hydrophobic amino acid) via its SH3 domain (Yun et al., 2007). Functional studies showed that Fyn increases 5-HT_6 receptor cell surface localization and, consequently, receptor-operated G protein signaling (Yun et al., 2007). Conversely, 5-HT_6 receptor activation triggers Fyn phosphorylation, as assessed by an increase in Tyr^{420} phosphorylation, an event contributing to 5-HT_6 receptor–induced activation of the Ras/Erk1/2 pathway (Fig. 32) (Yun et al., 2007). Whether 5-HT_6 receptor–mediated Fyn activation is involved in neuronal migration, attraction, and maturation of GABAergic synapses remains to be elucidated.

Further two-hybrid screens identified two other 5-HT_6 receptor–interacting proteins that control receptor trafficking. The first one is Jun activation domain–binding protein (Jab)1, a protein initially isolated as a c-Jun and Jun D binding partner that stabilizes their binding to activator protein 1 (AP-1) sites and potentiates them as transcription factors (Claret et al., 1996; Yun et al., 2010). Jab1 interacts with both i_3 loop and Ct of the receptor (Yun et al., 2010). Jab1 stabilizes surface expression of 5-HT_6 receptor and is necessary to maintain activity of endogenous receptors. In turn, 5-HT_6 receptor stimulation increases nuclear translocation of Jab1, suggesting that they may play a role in the regulation of gene expression via Jab1 (Fig. 32) (Yun et al., 2010). Furthermore, 5-HT_6 receptors and Jab1 are upregulated following middle cerebral artery occlusion–induced focal cerebral ischemia in rats. Likewise, exposure of cultured cells to hypoxic insults increased expression of both protein partners, which in turn reduce cell death induced by hypoxia (Fig. 32) (Yun et al., 2010). The second protein identified is light chain 1 (LC1) subunit of MAP1B protein (MAP1B-LC1), a microtubule-associated protein highly expressed in the brain (Kim et al., 2014). MAP1B-LC1 interacts with the Ct of the receptor and reduces receptor endocytosis, thereby increasing its cell surface expression and signal transduction activity (G_s coupling and Erk1/2 activation) (Kim et al., 2014).

The 5-HT_6 receptor i_3 loop recruits several proteins of the endocytic machinery, such as dynamin, AP-2, amphiphysin, and epsin as well as SNX14 (Ha et al., 2015). Like SNX27, SNX14 belongs to the sorting nexin family, predicted to have a role in protein sorting and vesicular trafficking (Carlton et al., 2005). It contains a putative RGS domain and a phox homology (PX) domain, an N-terminal hydrophobic region, and a PX-associated domain of unknown function. SNX14 is expressed at high levels in the nervous system (Carroll et al., 2001) and plays a critical role in both excitatory and inhibitory transmissions (Huang et al., 2014). SNX14 was found to inhibit 5-HT_6 receptor–dependent signaling via two different mechanisms: 1) it specifically binds to and sequesters $G\alpha_s$, thus inhibiting the G_s-cAMP pathway; and 2) it promotes 5-HT_6 receptor endocytosis and degradation (Fig. 32) (Ha et al., 2015). Furthermore, PKA phosphorylates SNX14 at two serine residues located in the RGS domain. SNX14 phosphorylation strongly reduces its affinity for $G\alpha_s$ and thereby prevents $G\alpha_s$ sequestration. Accordingly, phosphorylated SNX14 preferentially associates with 5-HT_6 receptor and can thereby enhance receptor internalization (Ha et al., 2015). Therefore, SNX14 has been considered as a dual, sequential, negative regulator of 5-HT_6 receptor–operated signaling, first by sequestering $G\alpha_s$ and second by inducing receptor endocytosis (Ha et al., 2015). Collectively, these findings indicate that 5-HT_6 receptor functional activity is finely modulated by its association with GIPs that exert contrasting effects on receptor trafficking and receptor-mediated signal transduction.

3. Recruitment of mTOR Complex 1 by 5-HT_6 Receptor: Potential Role in Cognitive Deficits Associated with Schizophrenia. Using a proteomics strategy combining coimmunoprecipitation of full-length receptor and tandem mass spectrometry, Meffre et al. (2012) identified 28 proteins that specifically associate with the 5-HT_6 receptor. This "receptosome" showed a remarkable enrichment in proteins of the mTOR pathway (Meffre et al., 2012). These include mTOR itself; Raptor (regulatory associated protein of TOR), an activator of mTOR that together with mTOR and proline-rich Akt substrate of 40 kDa constitutes the mTOR complex 1 (mTORC1); the small GTPase Rheb (Ras homolog enriched in brain), which directly activates mTOR when bound to GTP; the Tti1/Tel2 complex, required for assembly and stability of mTOR complexes; and the Ras GTPase activating protein neurofibromin, an upstream negative regulator of the pathway leading to mTOR activation (Fig. 33) (Meffre et al., 2012). This "receptosome" also includes Vps34, a protein of the class III PI3K family implicated in autophagosome formation (Bockaert and Marin, 2015). In line with this remarkable enrichment of the 5-HT_6 receptor complex in proteins of the mTOR pathway, agonist stimulation of the receptor results in the activation of the mTOR pathway both in a transfected cell line and in rodent brain, particularly in prefrontal cortex and striatum (Meffre et al., 2012). Notably, 5-HT_6 receptor–elicited mTOR activation depends on both its physical association with mTOR and the canonical PI3K/Akt/TSC/Rheb pathway involved in activation of mTORC1 by tyrosine kinase receptors (Fig. 33) (Meffre et al., 2012).

An overactivation of mTOR is observed in many genetic diseases in which mental retardation or

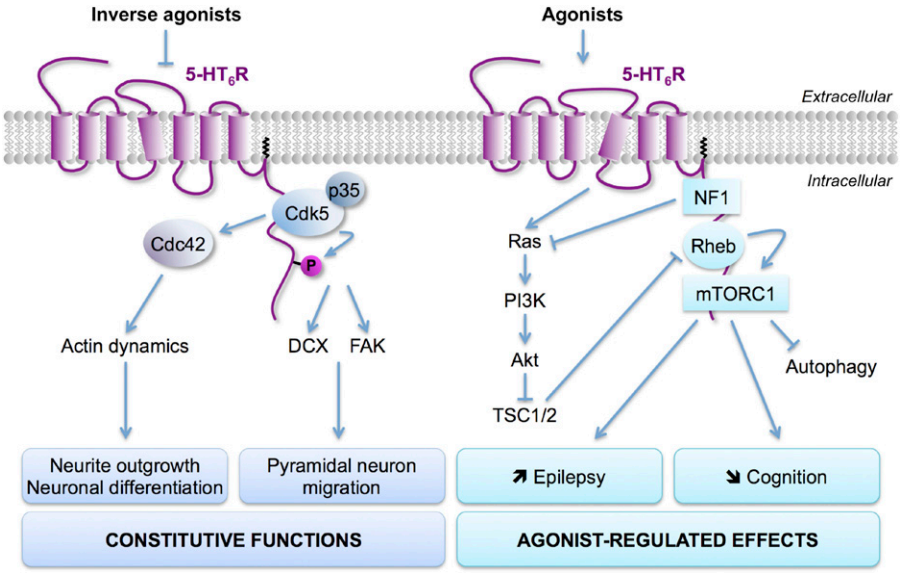

Fig. 33. Engagement of Cdk5 and mTOR signaling pathways by 5-HT_6 receptor and their role in neurodevelopmental processes and cognition. Left panel: The 5-HT_6 receptor constitutively interacts with Cdk5 (and its activator p35), which phosphorylates the receptor at Ser^{350}. This enables 5-HT_6 receptor to promote neurite growth via the activation of the Rho GTPase Cdc42. Cdk5 activity, under the control of 5-HT_6 receptor, also enables migration of pyramidal cortical neurons, likely via the phosphorylation of doublecortin (DCX) and focal adhesion kinase (FAK). These effects are agonist-independent and prevented by inverse agonists. Right panel: The 5-HT_6 receptor recruits several proteins of the mTOR pathway, including mTOR itself and Raptor, two protein components of the mTORC1 complex, neurofibromin (NF1), Vps34, and Rheb. Prefrontal 5-HT_6 receptors engage mTOR signaling upon agonist stimulation and in rat neurodevelopmental models of schizophrenia to compromise social cognition and episodic memory, whereas 5-HT_6 receptor blockade by antagonists or direct mTOR inhibition by rapamycin rescue cognitive deficits in these models.

cognitive deficits are observed [for a review, see Bockaert and Marin (2015)]. One classic example is tuberous sclerosis (TSC) caused by mutations in the *TSC1* or *TSC2* genes, which encode proteins of the TSC complex, namely, hamartin and tuberin, respectively. The TSC complex is the main GTPase activating protein for Rheb. Mutations in *TSC1* or *TSC2* result in inactivation of TSC, a process leading to nonphysiologic mTOR activation. About 50% of TSC patients show intellectual disabilities as well as deficits in memory, attention, and executive functions, and 20%–60% display ASD (Bockaert and Marin, 2015). Moreover, the mTORC1 inhibitor rapamycin rescues cognitive deficits in a mouse model of TSC ($Tsc2^{+/-}$ mice). Likewise, systemic administration of rapamycin prevents deficits in social cognition and novel object recognition induced by a 5-HT_6 receptor agonist in the rat (Meffre et al., 2012). In two neurodevelopmental rodent models of schizophrenia, the neonatal PCP model and the social isolation model (isolation after weaning), a sustained 5-HT_6 receptor–mediated mTOR activation that persists at the adult stage occurs specifically in prefrontal cortex (Meffre et al., 2012). Again, cognitive impairment observed in both models is reversed by an acute injection of rapamycin at the adult stage (Meffre et al., 2012). These findings suggest a critical role of mTOR activation not only in rare autism-related genetic disorders, such as TSC, but also in schizophrenia, a more frequent, multifactorial, and debilitating disorder. The mechanisms by which nonphysiologic mTORC1 activation induces cognitive deficits remain largely unknown. A recent report has shown that enhanced activity of mTORC1 in postmortem brain of ASD patients is associated with a decrease in autophagy, an increase in spine density, and a reduction of developmental spine pruning in layer V pyramidal neurons (Tang et al., 2014). Spine defects are likewise observed in $Tsc2^{+/-}$ mice (Bockaert and Marin, 2015). Whether inhibition of mTOR pathway contributes to the procognitive effects of 5-HT_6 receptor antagonists in patients with Alzheimer disease remains to be explored. Nonphysiologic mTOR activation, under the control of 5-HT_6 receptor, might also be involved in epilepsy. Indeed, an increase in 5-HT_6 receptor expression was found both in human epileptic tissue and the brain of rats treated with pilocarpine, a model of temporal lobe epilepsy (Wang et al., 2014). mTOR is also overactivated in the pilocarpine model, and a 5-HT_6 receptor antagonist prevents mTOR activation, increases latency of seizures, and reduces their severity (Wang et al., 2014). This suggests that blocking the 5-HT_6/mTOR pathway might be a valuable therapeutic strategy for epilepsy treatment.

4. Recruitment of Cyclin-Dependent Kinase 5 by 5-HT_6 Receptor: An Essential Step in Its Control of Neuronal Migration and Differentiation. The 5-HT_6 receptor is implicated in early steps of brain development, including neurulation and neuronal migration within the cortex (Jacobshagen et al., 2014; Dayer et al., 2015). Although the cAMP pathway contributes to the control of neuronal migration by 5-HT_6 receptors, a PKA inhibitor only partially reversed the effect of its stimulation upon neuronal migration (Riccio et al., 2009),

suggesting that other signaling pathways might be involved. Cyclin-dependent kinase (Cdk)5, which has been identified as a 5-HT_6 receptor partner by different AP-MS proteomics strategies (Fig. 33; Meffre et al., 2012; Duhr et al., 2014), was an obvious candidate. Indeed, Cdk5 is known to control actin cytoskeleton dynamics and various neurodevelopmental processes, such as neuronal migration (including migration of cortical pyramidal neurons), neurite growth, and synapse morphogenesis (Jessberger et al., 2009; Lalioti et al., 2010). Moreover, the 5-HT_6 receptor recruits, via its Ct, not only Cdk5 and its activator p35 but also a network of proteins functionally connected with Cdk5, including Wiskott-Aldrich syndrome protein–family verprolin homologous protein 1 (WAVE-1) and G protein inducer of neurite growth 1, two Cdk5 substrates; phosphatase 2A, which dephosphorylates and activates WAVE-1; and the Arp2/3 complex, which is also known to be activated by WAVE-1 (Duhr et al., 2014). Several lines of evidence indicate a role of Cdk5, under the control of the 5-HT_6 receptor, in the migration of pyramidal neurons: in utero electroporation of a 5-HT_6 receptor short hairpin RNA at E14.5 induces a mispositioning of these neurons, which can be rescued by electroporation of plasmids encoding wild-type 5-HT_6 receptor or 5-HT_6 receptors unable to couple to G_s or to bind to 5-HT (Jacobshagen et al., 2014). This indicates that the effect of 5-HT_6 receptors on pyramidal neuron migration is agonist-independent and is not mediated by the G_s-adenylyl cyclase pathway. Defect in migration elicited by silencing 5-HT_6 receptor expression is rescued by electroporation of plasmids expressing Cdk5 and its activator p35 (Jacobshagen et al., 2014). Moreover, 5-HT_6 receptor knockdown significantly reduces phosphorylation of doublecortin (at Ser^{297}) and focal adhesion kinase (at Ser^{732}) in primary cortical neurons, two Cdk5 substrates known to control migration of pyramidal neurons (Xie et al., 2003; Tanaka et al., 2004). Collectively, these findings indicate that 5-HT_6 receptors control migration of cortical pyramidal neurons through an agonist-independent, Cdk5-dependent mechanism (Jacobshagen et al., 2014; Dayer et al., 2015).

Beyond neuronal migration, the 5-HT_6/Cdk5 complex also controls neurite outgrowth and neuronal differentiation (Fig. 33). Its role in neurite growth was not only established in neuroblastoma-glioma NG108-15 cells, a commonly used cellular mode for investigating mechanisms underlying neuronal development, but also in primary neurons and brain explants (Duhr et al., 2014; Seo and Tsai, 2014). Reminiscent of its control of neuronal migration, the growth-promoting effects of the receptor is G_s- and agonist-independent. They are reversed by the selective 5-HT_6 receptor antagonist SB258585, which thus behaves as an inverse agonist in this model (Duhr et al., 2014). Likewise, SB258585 inhibits Cdk5 association with the 5-HT_6 receptor, indicating a specific recruitment of Cdk5 by a constitutively active receptor conformation. 5-HT_6 receptor–elicited neurite growth also depends on receptor phosphorylation at Ser^{350} by associated Cdk5. This suggests a reciprocal interplay between 5-HT_6 receptor and Cdk5, whereby the receptor stimulates Cdk5 activity and is itself a Cdk5 substrate. The signaling events downstream of the 5-HT_6/Cdk5 complex contributing to neurite growth have been partially characterized and involve Cdk5-dependent activation of the Rho GTPase cell division cycle (Cdc)42, a key regulator of actin cytoskeleton dynamics (Fig. 33) (Duhr et al., 2014). The precise mechanisms that control 5-HT_6 receptor/Cdk5 interaction, including the potential influence of other receptor partners, and the cellular events downstream of the 5-HT_6/Cdk5/Cdc42 pathway remain to be explored. As already mentioned, the regulatory role of Cdk5 during brain development extends to synapse formation. Whether 5-HT_6 receptor–dependent activation of Cdk5 controls synaptogenesis in addition to neuron migration and shaping also remains to be investigated.

XVIII. 5-HT Receptors and the Brain

A. Introduction

The diversity of 5-HT receptor signaling pathways and associated functional complexity in the brain is greater than any other tissue or organ. For historical perspectives on the contribution of neurophysiology, pharmacology, and molecular biology to the discovery of 5-HT receptor subtypes in the brain, see reviews in Bradley et al. (1986), Barnes and Sharp (1999), and Bockaert et al. (2010).

B. 5-HT Receptor Signaling in Neurons

All 5-HT receptor subtypes found in the brain are also found in the periphery except 5-ht_{1e}, 5-HT_{2C}, and 5-HT_6 receptors, for which there is little evidence for functional expression outside the CNS. The signaling properties of 5-HT receptors in the brain are identical to those in the periphery. Thus, the metabotropic 5-HT receptors couple to the three canonical signaling pathways, namely G_i, G_s, and $G_{q/11}$, which elicit the expected second messenger cascades, and this is proven to occur in neurons in most cases.

The G_i-coupled 5-HT receptors that inhibit adenylyl cyclase and cause a fall in cAMP encompass the 5-HT_1 receptor family, comprising 5-HT_{1A}, 5-HT_{1B}, 5-HT_{1D}, 5-ht_{1E}, and 5-HT_{1F} subtypes. Evidence suggests that the 5-HT_5 receptor family, 5-HT_{5A} and 5-ht_{5b}, is also G_i-coupled, although, to date, this has only been demonstrated in cultured cells and not native neurons. Excellent reviews on the signaling (Hannon and Hoyer, 2008; Bockaert et al., 2010; McCorvy and Roth, 2015) and consequent electrophysiological (Lamb and Aghajanian, 2006; Andrade and Beck, 2010; Marek,

2010) properties of 5-HT_1 receptors can be found elsewhere.

Independent of the involvement of a decrease in cAMP, 5-HT_1 receptors also open GIRKs to hyperpolarize neurons and inhibit the opening of voltage-gated calcium channels; this is best understood for 5-HT_{1A} receptors (e.g., Montalbano et al., 2015). 5-HT_1 receptors are also likely to elicit other (noncanonical) G protein–dependent signals, including via ERK and Akt kinase, again as exemplified by studies of the 5-HT_{1A} receptor. Adding further complexity, it is well established that 5-HT_{1A} receptors have different properties depending on their pre- or postsynaptic localization. In particular, agonists tend to have greater efficacy at presynaptic 5-HT_{1A} receptors, and the latter have greater tendency to desensitize than postsynaptic 5-HT_{1A} receptors. The reasons for these differences are uncertain but, as discussed elsewhere (Clarke et al., 1996; Barnes and Sharp, 1999; Mannoury la Cour et al., 2006; Garcia-Garcia et al., 2014), could relate to presynaptic versus postsynaptic differences in 5-HT_{1A} receptor reserve, efficiency of G protein coupling, non–G protein–dependent signaling, or even biased agonism—that is, the generation of signals that are ligand-dependent. Regarding the latter, emerging data strongly support the view that selective 5-HT_{1A} receptor agonists can preferentially direct receptor signaling to particular intracellular pathways in a brain region–specific manner (Becker et al., 2016).

The 5-HT_2 receptor family, 5-HT_{2A}, 5-HT_{2B}, and 5-HT_{2C}, comprise $G_{q/11}$-coupled receptors that activate phospholipase C, leading to increased formation of inositol trisphosphate and diacylglycerol and then mobilization of intracellular calcium and PKC activation. 5-HT_2 receptor stimulation also results in neuronal excitation in a variety of brain regions, likely involving the closure of potassium channels. A 5-HT_2 receptor–mediated increase in excitatory postsynaptic currents is also often observed, which is thought to be mediated either directly or indirectly through increased release of glutamate (Lambe and Aghajanian, 2006; Marek, 2010).

It is now recognized that 5-HT_2 receptor signaling goes well beyond activation of the phospholipase C/PKC pathway. For instance, noncanonical signaling pathways associated with 5-HT_{2A} receptor activation include phospholipase A_2, the ERK pathway, and signaling via small G proteins (e.g., Rho A and Rab4), and there are similar such findings for 5-HT_{2B} and 5-HT_{2C} receptors [for review, see Bockaert et al. (2010), Halberstadt (2015), McCorvy and Roth (2015), Maroteaux et al. (2017)]. Interesting findings are emerging regarding noncanonical signaling by 5-HT_2 receptors. Specifically, the 5-HT_{2C} receptor is able to activate ERK via a G protein–independent mechanism through recruitment of calmodulin and β-arrestin (Labasque et al., 2008). Furthermore, recent studies on noncanonical signaling by the 5-HT_{2B} receptor found evidence of ligand-dependent signals and then used the crystal structure of the receptor to identify high-resolution, structural basis for the biased signaling (Wacker et al., 2013, 2017a,b). This evidence of ligand-dependent signaling through 5-HT_2 receptors has relevance to the psychotropic effects of 5-HT_{2A} receptor agonists, some of which are hallucinogenic but not all (Gonzalez-Maeso et al., 2007), and may also explain the long-lasting effects of LSD that are difficult to explain on the basis of pharmacokinetics alone (Wacker et al., 2017). However, the signaling pathways mediating the characteristic effects of hallucinogens have not been identified conclusively. Biased agonism at 5-HT_2 receptors offers intriguing possibilities for therapeutic potential (e.g., avoidance of hallucinogenic properties), although this is yet to be explored, keeping in mind that 5-HT_{2A} receptor agonists have profound effects on vascular and other smooth muscle in addition to their psychotropic effects. Also, findings of the existence of constitutive activity at 5-HT_{2A} and 5-HT_{2C} receptors, and 5-HT_2 receptor ligands with inverse agonist properties, provides further avenues for 5-HT_2 receptor drug development (e.g., Aloyo et al., 2009).

The remaining metabotropic 5-HT receptors to be considered, specifically 5-HT_4, 5-HT_6, and 5-HT_7 receptors, are G_s coupled and thus activate adenylyl cyclase to increase cAMP. At the electrophysiological level, 5-HT_4 receptor activation on neurons is associated with a slow membrane depolarization mediated by a reduction of a slow after-hyperpolarization potential and facilitation of L-type calcium channels through protein kinase A activation (Andrade and Chaput, 1991; Birnstiel and Beck, 1995). Similarly, the neuronal 5-HT_7 receptor is also frequently associated with a direct depolarizing or excitatory effect, also likely mediated by actions on slow after-hyperpolarization and L-type calcium channels (Bacon and Beck, 2000; Andrade, 2006).

As with the other metabotropic 5-HT receptors, the 5-HT_4 receptor generates a diversity of signals independent of the second messenger. In addition, both the 5-HT_4 and 5-HT_6 receptors provide further examples of metabotropic 5-HT receptors that are capable of noncanonical signaling. For example, 5-HT_4 receptor stimulation activated ERK in cultured cells and neurons in a manner that was G protein–independent (G_q, G_i, G_o) but dependent on Src tyrosine kinase (Barthet et al., 2007; Bockaert et al., 2010), and there are analogous observations for 5-HT_6 receptors (Yun et al., 2007).

As a complement to the slow signaling elicited by metabotropic 5-HT receptors, the ionotropic 5-HT_3 receptor mediates rapid synaptic transmission. All five human 5-HT_3 receptor genes (*5-HT3A-3E*) are homologous with other members of the Cys-Cys loop ligand-gated channel superfamily (e.g., nicotinic, $GABA_A$, glutamate, or glycine receptors) (Barnes et al., 2009). Although there is uncertainty regarding the composition of the native 5-HT_3 receptors in the brain, pentameric coassemblies of 5-HT_{3A} and 5-HT_{3B} subunits is

a likely common occurrence [for reviews, see Niesler (2011) and Thompson and Lummis (2013)]. Allosteric modulators of the 5-HT_3 receptor are emerging to add to the possibilities for 5-HT_3 receptor manipulation for therapeutic benefit (e.g., Trattnig et al., 2012 and Newman et al., 2013).

Overall, the complexity of neuronal signaling events elicited by 5-HT receptors is yet to be fully revealed. The metabotropic 5-HT receptors are clearly capable of both G protein–dependent and G protein–independent signaling, and it is also clear that the diverse signals generated by these receptors can be biased according to the agonist used. Moreover, as covered elsewhere, further complexity arises from the large number of protein partners influencing cellular localization and trafficking as well as signal tuning of 5-HT receptors, their homo- or heterodimerization with other GPCRs, and the alternative RNA splicing and editing of certain 5-HT receptors (especially 5-HT_{2C}, 5-HT_4, and 5-HT_7 receptors) to generate variability in function (Hannon and Hoyer, 2008; Bockaert et al., 2010; McCorvy and Roth, 2015).

C. Expression of 5-HT Receptors in the Brain

Application of a combination of techniques such as receptor autoradiography, in situ hybridization, and immunocytochemistry has revealed maps of 5-HT receptor subtype distribution in the brain that are largely similar across a range of vertebrate species (although of note, the 5-ht_{1e} receptor is not expressed in rodents, forebrain 5-HT_3 receptors are differentially expressed across species, and the full-length 5-ht_{5b} receptor is not expressed in man). The use of BAC transgenic mice engineered to express a fluorescent or colored reporter genes under the control of specific 5-HT receptor promoters, such as in the case of 5-HT_{2A} receptor (Weber and Andrade, 2010), reveals further anatomic detail of 5-HT receptor distribution. Although the 5-HT receptor maps are largely based on data collected from the rodent brain, a high-resolution PET atlas of four 5-HT receptors (5-HT_{1A}, 5-HT_{1B}, 5-HT_{2A}, and 5-HT_4 receptors) and the 5-HT transporter was recently described for the human brain (Beliveau et al., 2017). It is now clear from these mapping studies that each 5-HT receptor subtype has a unique distribution pattern in the brain but one that often overlaps with that of other 5-HT receptor subtypes. These differential distribution patterns suggest that different 5-HT receptor subtypes are likely associated with distinct CNS functions amenable to manipulation using 5-HT receptor subtype selective pharmacological agents.

D. Presynaptic 5-HT Receptors

Two of the 14 5-HT receptors are highly expressed presynaptically (i.e., expressed by 5-HT neurons themselves). Thus, 5-HT_{1A} receptors are located on the soma and dendrites of 5-HT neurons, whereas 5-HT_{1B} receptors are expressed at the soma but then trafficked down the axons to the terminals (Riad et al., 2000). It is firmly established from both in vitro and in vivo models that 5-HT_{1A} and 5-HT_{1B} receptors function as autoreceptors and exert direct inhibitory control over the firing of 5-HT neurons and terminal 5-HT release, respectively. Thus, studies over 40 years ago finding that 5-HT itself, or nonselective 5-HT agonists such as LSD, inhibited the efflux of preloaded radiolabeled 5-HT from rodent brain slice preparations were followed up by extensive pharmacological analysis that identified that this effect was mediated by 5-HT_{1B} receptors located on 5-HT nerve terminals (Gothert and Weinheimer, 1979; Mounsey et al., 1982; Engel et al., 1986; Fink and Gothert, 2007). Similarly detailed pharmacological studies using electrophysiological recordings of 5-HT neurons and in vivo microdialysis measurements of 5-HT release characterized the autoreceptor function of somatodendritic 5-HT_{1A} receptors (e.g., Vandermaelen and Aghajanian, 1983; Sharp et al., 1989; and Adell and Artigas, 1998).

There are more recent reports of the presence of other 5-HT receptor subtypes in the midbrain raphe nuclei, particularly 5-HT_{1D}, 5-HT_{1F}, 5-HT_{2B}, 5-HT_{2C}, and 5-HT_{5A}. However, with the exception of 5-HT_{2C} (located on raphe GABAergic interneurons and not 5-HT neurons), expression levels of these receptors in the midbrain raphe are low, and their functional significance in terms of 5-HT neuron control is not certain. Nevertheless, recent findings demonstrate that feedback control of 5-HT neurons is not likely limited to 5-HT_1 autoreceptors; instead, it includes 5-HT receptors located on postsynaptic targets that have the physiologic effects of 5-HT autoreceptors but use additional 5-HT receptor subtypes and operate via neural inputs to 5-HT neurons. For example, evidence supports a role for postsynaptic 5 HT_{1A}, 5-HT_{2A}, and 5-HT_{2C} receptors in the inhibitory control of 5-HT neurons, whereas 5-HT_4 and 5-HT_6 receptors are excitatory in this regard (Ge and Barnes, 1996; Sharp et al., 2007; Sharp, 2010; Brouard et al., 2015). Many of these feedback pathways appear to be localized on cortical pathways projecting back to the midbrain raphe nuclei (5-HT_{1A}, 5-HT_{2A}, 5-HT_4, and 5-HT_6 receptors), although contributions from non–5-HT neurons such as habenula inputs to the raphe (5-HT_{2C} receptors) further emphasize the complexity of 5-HT neuron control.

E. Postsynaptic 5-HT Receptors

5-HT receptor mapping studies demonstrate that the majority of 5-HT receptor subtypes are located postsynaptically (i.e., expressed by non–5-HT neurons, sometimes referred to as heteroceptors, including the receptor subtypes involved in feedback control of 5-HT neurons) and that each 5-HT receptor subtype has an expression pattern that is distinct but often overlaps with that of other 5-HT receptors. Even 5-HT receptors

from the same family have different CNS distributions (e.g., 5-HT_{2A} vs. 5-HT_{2C} and 5-HT_{1A} vs. 5-HT_{1B}). Some 5-HT receptors are expressed at higher levels than others; in particular, 5-HT_{1A} and 5-HT_{2A} receptors are among the most abundant, whereas in comparison, levels of 5-HT_{1D}, 5-ht_{1E}, 5-HT_{1F}, 5-HT_{2B}, and 5-HT_{5A} receptors are much less abundant. Detailed maps of 5-HT receptor binding sites and protein and mRNA distribution in the brain are reported in a number of review articles (Mengod et al., 2006, 2010; Palacios, 2016).

At the macrolevel, and in keeping with the widespread 5-HT innervation of the brain, 5-HT receptors of different types are expressed in many brain regions that play key roles in numerous CNS functions. For example, regions rich in 5-HT receptors include cortical and limbic areas (5-HT_{1A}, 5-$HT_{2A/2C}$, 5-HT_3, 5-HT_4, and 5-HT_6 receptors), the basal ganglia (5-HT_{1B}, 5-HT_4, and 5-HT_6 receptors), mesolimbic pathways (5-HT_{1B}, 5-$HT_{2A/2C}$, and 5-HT_4 receptors), hypothalamus (e.g., paraventricular and arcuate nuclei; 5-HT_{2C} receptor), suprachiasmatic nucleus (5-HT_7 receptor), trigeminal nucleus (5-$HT_{1B/1D}$ receptors), dorsal vagal complex (encompassing the area postrema and nucleus tractus solitarus; 5-HT_3 receptor), and the spinal cord (dorsal root ganglia; 5-$HT_{2B/2C}$ and 5-HT_3 receptors). This list depicting 5-HT receptor distribution is of course not exclusive and does not take into account the relative density of receptors in the different regions [e.g., in many regions, 5-HT_{2C} receptors are present at much lower densities than 5-HT_{2A} receptors (with the remarkable exception of the choroid plexus), and this is also the case for 5-HT_{1D} compared with 5-HT_{1B} receptors].

F. Cellular Localization of Central Nervous System 5-HT Receptors

The principal cellular location of CNS 5-HT receptors is neurons; indeed, evidence for the expression of 5-HT receptors by adult native nonneural cells such as glial cells is, at best, inconsistent. For example, reports of glial cell expression of 5-HT_{1A} and 5-HT_{2A} receptors appear to be dependent on the antibody used. However, emerging evidence suggests that activated microglia may express 5-HT_{2B} and other 5-HT receptors (Krabbe et al., 2012; Kolodziejczak et al., 2015). Nevertheless, 5-HT receptors are undoubtedly present in cells of the cerebrovasculature, as most evident for 5-HT_{1B} receptors (Riad et al., 1998).

On the whole, the expression of specific 5-HT receptors is not restricted to particular neuron types. For example, among the complex microcircuitry of the cerebral cortex, hippocampus, and amygdala, 5-HT_{1A} and 5-HT_{2A} receptors are expressed both on pyramidal neurons (glutamatergic) and certain classes of GABAergic interneurons (particularly those expressing parvalbumin). Elsewhere in the brain, 5-HT_{1A} receptors are localized on 5-HT neurons in the raphe nuclei (see above) and cholinergic neurons in the septum, whereas 5-HT_{2A} receptors are expressed by midbrain dopamine neurons. On the other hand, 5-HT_3 receptors in rodent cortex and hippocampus mark a specific population of GABA interneurons with distinct chemical, morphologic, and anatomic properties (Lee et al., 2010), and these receptors are also present in pyramidal neurons, particularly in humans (Brady et al., 2007). In comparison, cortical 5-HT_6 receptors are preferentially (although not exclusively) expressed by pyramidal cells (Helboe et al., 2015).

Not surprisingly, there are multiple interactions between 5-HT and other neurotransmitter systems, and particular attention has been paid to 5-HT interactions with the other monoamines, noradrenaline and dopamine. These interactions are highly complex, involving multiple 5-HT receptor subtypes located at pre- and postsynaptic sites within complicated, overlapping circuitry, such that prediction of the overall effect of 5-HT is often not possible. However, at the level of individual receptors, the rules can be more straightforward. For example, the distribution of 5-HT_{2A} and 5-HT_{2C} receptors within the mesolimbic dopamine system has allowed the general consensus that 5-HT_{2A} receptors stimulate and 5-HT_{2C} receptors inhibit dopamine transmission (Howell and Cunningham, 2015).

Given the differential expression patterns of the 5-HT receptors, it is highly unlikely that all 14 5-HT receptors are expressed by a single neuron. Evidence from earlier double- and triple-labeling in situ hybridization studies support the idea that a single postsynaptic synapse may express a combination of two to three 5-HT receptors (Mengod et al., 2010). Studies combining whole-cell patch clamp recordings with single-cell reverse transcriptase polymerase chain reaction (RT-PCR) to characterize 5-HT receptor expression are in keeping with this idea. Thus, analysis of the 5-HT receptor mRNA content of neurons of the bed nucleus of the stria terminalis revealed that individual neurons could be subdivided according to the prominent expression of two to three distinct 5-HT receptor transcripts in largely excitatory/inhibitory combinations (e.g., 5-HT_{1A}/5-HT_7, 5-HT_3/5-HT_7, 5-HT_{1B}/5-HT_4, 5-HT_{1A}/5-HT_{1B}/5-HT_{2A}, and 5-HT_{1A}/5-HT_{2A} receptors) that matched electrophysiological observations (Hazra et al., 2012). A similar analysis of the 5-HT receptor mRNA content of single neurons of the preoptic nucleus revealed two types of neurons that predominantly expressed a combination of inhibitory (5-HT_{1A}) and excitatory (5-HT_{2C}, 5-HT_4, 5-HT_7) 5-HT receptors, whereas others expressed excitatory 5-HT receptors alone, again in keeping with electrophysiological findings (Sangare et al., 2016). This evidence of 5-HT receptor expression conferring both excitatory and inhibitory signaling effects of 5-HT at the single-cell level is born out more generally in electrophysiological experiments recording the effects of either bath application of 5-HT or electrical and optogenetic

activation of 5-HT neurons (Andrade, 2006; Andrade and Beck, 2010; Sengupta et al., 2017).

Generally speaking, there is a very good match between 5-HT receptor mRNA and protein (e.g., Mengod et al., 2010), suggesting that the majority of 5-HT receptor proteins are not trafficked significantly along axons and reside largely at the levels of the neuronal soma and dendrites. Notable exceptions to this rule are 5-HT_{1B} and 5-HT_3 receptors. The former are trafficked either to nerve terminals of 5-HT neurons where they function as autoreceptors or to nerve terminals of other neurons and especially GABA neurons where they modulate GABA release. On the other hand, it is estimated that about 70%–80% of the 5-HT_3 receptors are located on nerve endings, where their role is also to modulate neurotransmitter release (e.g., dopamine, cholecystokinin, glutamate, acetylcholine, and GABA) (Hannon and Hoyer, 2008; Walstab et al., 2010). A final point of note is that, unusually, 5-HT_6 receptors are expressed on neuronal primary cilia (Hamon et al., 1999), the functions of which remain obscure.

At the ultrastructural level, electron microscopy studies show that all metabotropic 5-HT receptors visualized thus far are located extrasynaptically, which has reinforced the idea that 5-HT principally signals via volume transmission (Descarries et al., 2006). However, synaptic localization of 5-HT receptors cannot be excluded, as these are challenging studies that are often limited by the properties and specificity of available antibodies. Moreover, it is beyond doubt that 5-HT is present in vesicles that are synaptic as well as nonsynaptic. An additional complexity in signaling at 5-HT synapses is cotransmission, with coreleased glutamate emerging as a key player (e.g., Sengupta et al., 2017).

G. Behavioral Roles of 5-HT Receptors in the Brain

1. Introduction. Knowledge of the regional and cellular location of 5-HT receptors in the brain has been immensely helpful in understanding a vast literature on the behavioral effects of pharmacological and genetic manipulation of 5-HT receptors. Collectively, these developments are casting light on the likely behavioral functions of 5-HT receptors. So far, no behavioral response can be confidently ascribed to activation of the CNS 5-HT_{1D}, 5-ht_{1e}, 5-HT_{1F}, or 5-HT_5 receptors.

2. 5-HT_1 Receptor Family.

a. 5-HT_{1A} receptors. The behavioral functions of 5-HT_{1A} receptors have been the focus of much research given the widespread and high CNS expression of these receptors, combined with the availability of a large number of selective 5-HT_{1A} receptor ligands. It has long been known that in rodents, administration of 8-OH-DPAT and other 5 HT_{1A} receptor agonists causes a wide range of behavioral and physiologic effects, including changes in motor function (especially induction of the 5-HT behavioral syndrome), hyperphagia, hypothermia, altered sexual behavior, and changes in pain threshold. Also, there is a large literature demonstrating that 5-HT_{1A} receptor agonists have antidepressant and anxiolytic activity and influence a range of cognitive domains relevant to symptoms of schizophrenia (for reviews, see Traber and Glaser, 1987; Handley, 1995; and Newman-Tancredi, 2010). These findings accord with preclinical and clinical evidence implicating changes in 5-HT_{1A} receptors in the pathophysiology of a variety of psychiatric illnesses, including depression, anxiety and other stress-related disorders, and schizophrenia. Appropriate 5-HT_{1A} receptor function also appears critical for antidepressant drug efficacy (Richardson-Jones et al., 2010; Samuels et al., 2016). The diverse behavioral effects of 5-HT_{1A} receptor agonists are likely to involve an action at 5-HT_{1A} receptors in multiple forebrain and midbrain sites; the contribution of presynaptic and postsynaptic 5-HT_{1A} receptors to specific behavioral effects of 5-HT_{1A} receptor agonists is not always certain, and indirect effects on other transmitter systems, particularly noradrenaline and dopamine, may often be involved.

Despite these complexities, a significant body of evidence links the decrease in 5-HT transmission evoked by 5-HT_{1A} autoreceptor activation to anxiolytic effects, whereas an increase in 5-HT transmission evoked by activation of postsynaptic 5-HT_{1A} receptors is associated with antidepressant effects [see Barnes and Sharp (1999) and *II. 5-HT_{1A} Receptor* in the present review]. Much of the evidence for this has come from psychopharmacological studies in animals and humans, and it is being increasingly reinforced by studies using advanced genetic mouse models that have the power to make targeted manipulation of pre- versus postsynaptic 5-HT_{1A} receptors. In particular, genetic mouse constructs with selective knockdown of 5-HT_{1A} autoreceptor expression generate an increase in anxiety phenotypes (Richardson-Jones et al., 2011). In contrast, selective knockdown of postsynaptic 5-HT_{1A} receptors is associated with depressive-like phenotype, suggesting a differential impact of pre- versus postsynaptic 5-HT_{1A} receptors on anxiety and depression mechanisms. Paradoxically, although these phenotypes associated with genetic manipulation can be reproduced by pharmacological means, they are only observed when 5-HT_{1A} receptor suppression is initiated in early life and not during adulthood. Thus, these data point to a developmental mechanism, that is, altered pre- and postsynaptic 5-HT_{1A} receptor signaling during development, causing an adult behavioral phenotype through impacting the normal formation of anxiety/depression circuitry (Richardson-Jones et al., 2011; Garcia-Garcia et al., 2014, 2016). An analogous theory has been posited to explain the behavioral phenotype of 5-HT transporter mutant mice (Ansorge et al., 2004). Consideration of developmental mechanisms may therefore be appropriate when interpreting the behavioral phenotype of other mouse models with

altered 5-HT receptor expression (Gingrich et al., 2003; Berger and Tecott, 2006; O'Leary and Cryan, 2010).

Aside from the interesting picture emerging through genetic models, there is increasing evidence that behavioral effects of 5-HT_{1A} agonist administration are dependent on the agonist used. Divergent effects of certain 5-HT_{1A} agonists have been reported across a range of models of cognition and emotional behavior as well as in relevant neurochemical and neurophysiological paradigms. These findings raise the possibility that different behaviors are being evoked by different signals because of biased agonism, and differential sensitivity of pre- and postsynaptic 5-HT_{1A} receptors to the agonists may be a contributing factor. Thus, functionally and anatomically distinct subpopulations of 5-HT_{1A} receptors, combined with an emerging diversity of biased 5-HT_{1A} receptor agonists, forecasts new categories of 5-HT_{1A} drugs for selective behavioral manipulation and thereby potential multiple therapeutic applications.

b. 5-HT_{1B} receptors. Preclinical studies on the behavioral effects of the 5-HT_{1B} receptor ligands have been hampered by the lack of drug tools with sufficient selectivity or brain penetration, and this continues to be somewhat problematic in the case of 5-HT_{1B} receptor agonists. Early studies on the behavioral effects of 5-HT_{1B} receptor agonists and antagonists in rodents have been extensively reviewed (Lucki, 1992; Middlemiss and Tricklebank, 1992; Barnes and Sharp, 1999). These earlier findings, combined with more recent preclinical and clinical studies, emphasize a role for 5-HT_{1B} receptors in depression and anxiety behaviors on the one hand [for review, see Ruf and Bhagwagar (2009) and Fakhoury (2016)] and aggression and impulse control on the other [for review, see Nautiyal et al. (2015)]. Moreover, the role of pre- versus postsynaptic 5-HT_{1B} receptors in these behaviors is beginning to be addressed using tissue-specific and time-dependent conditional 5-HT_{1B} receptor mutant mice.

Evidence from earlier neuropharmacological studies suggests on the whole that 5-HT_{1B} receptor agonists have antidepressant effects in animal models, whereas 5-HT_{1B} receptor antagonists are anxiolytic (Fakhoury, 2016). The findings with 5-HT_{1B} receptor antagonists are to some extent consistent with evidence that mice with a global 5-HT_{1B} receptor knockout demonstrate anxiolytic and antidepressant-like phenotypes (Mayorga et al., 2001; Jones and Lucki, 2005; Bechtholt et al., 2008). Moreover, it is reported these phenotypic effects can be recapitulated in genetic mice with selective loss of 5-HT_{1B} autoreceptors (Nautiyal et al., 2015). The latter group argue that relative to 5-HT_{1B} autoreceptors, postsynaptic 5-HT_{1B} receptors may have an opposing effect on anxiety and depressive behaviors, thereby potentially accounting for the divergent actions of 5-HT_{1B} receptor agonists and antagonists noted above. Importantly, an anxiolytic and antidepressant-like phenotype was generated by knockout of 5-HT_{1B} autoreceptors in adulthood and not during early postnatal life, thereby making a developmental mechanism unlikely (Nautiyal et al., 2015). Collectively, these data suggest that drugs that selectively block 5-HT_{1B} autoreceptors may be useful for the treatment of anxiety and depression. Currently, there are no drugs with this property, although developments with biased 5-HT_{1A} receptor agonists (see above) offer the potential for such agents in the future.

Another interesting phenotype of mice with a global 5-HT_{1B} receptor knockout is increased aggression and impulsivity. This finding fits in with earlier evidence that certain 5-HT_{1B} receptor agonists ("serenics") have antiaggressive properties (Olivier et al., 1995). Moreover, these data fit with a consistent line of evidence associating reduced brain 5-HT transmission with high levels of impulsivity and aggression. The recently available conditional 5-HT_{1B} receptor knockout mouse has allowed for further examination of the role of pre- versus postsynaptic 5-HT_{1B} receptors in aggression and impulsivity (Nautiyal et al., 2015). It was found that heightened aggression and impulsivity was linked to loss postsynaptic 5-HT_{1B} receptors and not 5-HT_{1B} autoreceptors. However, although the increase in aggression involved a development mechanism (i.e., recapitulated by early life knockout), the increase in impulsivity was separable and was linked to loss of postsynaptic 5-HT_{1B} receptors in adulthood. These data raise the possibility that pharmacologic agents targeting 5-HT_{1B} receptors may be therapeutically effective in disorders associated with loss of impulse control. At least in the case of 5-HT_{1B} receptor agonists, these agents have been widely used in the clinic since the first development of sumatriptan for the acute treatment of migraine (Humphrey, 2008). It may be argued at length whether the effects of triptans in migraine involve a neuronal component versus vascular/inflammatory mechanisms (Humphrey and Goadsby, 1994) and whether triptans are considered safe in terms of CNS adverse effects.

3. 5-HT_2 Receptor Family. The behavioral effects of 5-HT_2 receptor agonists in rodents are many, ranging from changes in both unconditioned (e.g., head-twitches, increased motor activity, hypophagia, and hyperthermia) and conditioned responses (e.g., punished responding and drug discrimination) [for review, see Koek et al. (1992), Barnes and Sharp (1999), and Halberstadt (2015)]. It has become possible to identify the role of the different 5-HT_2 receptor subtypes in these behaviors with some degree of confidence through the availability of highly selective antagonists (for each of 5-HT_{2A}, 5-HT_{2B}, and 5-HT_{2C} receptors) and agonists (especially 5-HT_{2C} receptor agonists) as well as mutant mice with the different receptors selectively knocked out [for review, see Berger and Tecott (2006), O'Leary and Cryan (2010), Halberstadt (2015), and Di Giovanni and De Deurwaerdere (2016)].

a. 5-HT$_{2A}$ receptors. It is clear from detailed pharmacological analysis that the 5-HT$_{2A}$ receptor mediates the effects of serotonergic hallucinogens such as LSD and psilocybin in various behavioral models in animals, including drug discrimination, the head-twitch response, and locomotion (Halberstadt, 2015). The evidence is equally strong that the 5-HT$_{2A}$ receptor mediates the hallucinogenic effects of these drugs in humans. Prior to their prohibition in the late 1960s, psychedelic drugs such as LSD and psilocybin were used extensively in the treatment of major depression as well as anxiety-related disorders and addictions, and results were generally encouraging. Despite the restrictions that remain in place around these agents, there has been renewed interest in their use for experimental medicine studies and therapeutic purposes. Psilocybin in particular has been subject to investigation in a range of human psychopharmacological and brain imaging studies, which have contributed proof-of-principle and dose/safety data for clinical trials (Nutt, 2016). Moreover, recent clinical data demonstrate that acute administration of psilocybin (combined with psychologic support) causes a sustained lowering of depression and anxiety ratings in cancer patients (Griffiths et al., 2016; Ross et al., 2016) and treatment resistant-depression (Carhart-Harris et al., 2016), which is likely to encourage further studies in these and other patient groups.

The potent 5-HT$_{2A}$ receptor antagonist properties of many second-generation antipsychotics has been linked to the reported superior antipsychotic efficacy and reduced side-effect profile of these agents compared with early drugs (Meltzer and Massey, 2011). Although selective 5-HT$_{2A}$ receptor blockade is unlikely to be sufficient to generate a useful antipsychotic effect per se, evidence that the 5-HT$_{2A}$ receptor inverse agonist pimavanserin has antipsychotic actions in relevant animal models (Vanover et al., 2006) and in certain patient populations (Fox, 2014) suggests a novel alternative way to treat psychosis.

Recent reports that 5-HT$_{2A}$ receptor antagonists augment the effect of 5-HT uptake inhibitors in preclinical models (Marek et al., 2003, 2005; Boothman et al., 2006) may link to evidence that drugs with 5-HT$_{2A}$ receptor antagonist properties are helpful as augmenting agents in treatment-resistant depression (Marek et al., 2003). Although the mechanism behind this effect of 5-HT$_{2A}$ receptor blockade is not certain, it may link to evidence of an inhibitory 5-HT$_{2A}$ receptor–mediated feedback on 5-HT neurons (Sharp et al., 2007). 5-HT$_{2A}$ receptor blockade has also been associated with impulse control; thus, selective 5-HT$_{2A}$ receptor antagonists decrease impulsivity in animal models (Winstanley et al., 2004; Winstanley, 2011), and there is support for this action from psychopharmacological studies in humans (Rock et al., 2013). Interestingly, both lithium and the organoselenium compound, ebselen, which like lithium blocks signaling through phosphoinositide pathway, inhibit 5-HT$_{2A}$ receptor function, and both agents reduce impulsivity in animal models and humans (Singh et al., 2013; Masaki et al., 2016). This raises the possibility that 5-HT$_{2A}$ receptor antagonists/inverse agonists as well as novel lithium mimetics may have utility in the control of disorders of impulse control.

b. 5-HT$_{2B}$ receptors. The combination of low 5-HT$_{2B}$ receptor expression in the brain and the lack of selective 5-HT$_{2B}$ receptor ligands has hampered progress in establishing whether these receptors are able to elicit robust behavioral effects. This situation has moved forward with the development of 5-HT$_{2B}$ knockout mice, which have striking phenotypes across a range of modalities, including deficits in sensorimotor gating, social interaction, attention, learning, and memory as well as elevated impulsivity and altered sleep patterns (Pitychoutis et al., 2015). Furthermore, some of these effects (sleep and sensorimotor gating) can be phenocopied by administration of a selective 5-HT$_{2B}$ receptor antagonist. However, 5-HT$_{2B}$ knockout is also associated with severe cardiac abnormalities and embryonic and postnatal lethality, which might lead to neurodevelopment issues in surviving animals that could confound the interpretation of these adult CNS phenotypes (as noted above). Although studies involving conditional 5-HT$_{2B}$ knockout strategies and further pharmacological phenocopying are awaited, these data raise interesting possibilities regarding the functional role of CNS 5-HT$_{2B}$ receptors. As noted elsewhere (Hutcheson et al., 2011), 5-HT$_{2B}$ receptor agonism as a therapeutic approach is fraught by clinical evidence that such agents may induce valvulopathies, pulmonary hypertension that can have lethal consequences; thus, many therapeutic agents with 5-HT$_{2B}$ receptor agonist properties (e.g., fenfluramine, pergolide, and cabergoline) have now been withdrawn from the market (Hutcheson et al., 2011). Nevertheless, recent data showing biased agonism at the 5-HT$_{2B}$ receptor (Wacker et al., 2013) offers the potential for future 5-HT$_{2B}$ receptor agonists that may be devoid of these systemic adverse effects.

c. 5-HT$_{2C}$ receptors. Early studies recognized that several behavioral responses were likely associated with activation of central 5-HT$_{2C}$ receptors, including hypolocomotion, hypophagia, anxiety, penile erection, and hyperthermia [for review, see Barnes and Sharp (1999)]. Initially, these associations were largely based on observations using nonselective 5-HT$_{2C}$ receptor ligands. However, with increased availability of 5-HT$_{2C}$ receptor–selective compounds and the creation of 5-HT$_{2C}$ knockout mice, evidence for the involvement of the 5-HT$_{2C}$ receptor in many of these responses is now compelling [for review, see Di Giovanni and De Deurwaerdere (2016)]. Moreover, these developments expanded the CNS processes likely to be under 5-HT$_{2C}$ receptor control to include various behaviors and cognitions linked to compulsive drug- and food-seeking as well as the central control of energy homeostasis,

oral dyskinesia, wakefulness, and even control seizure threshold.

In the last decade or so, there has been considerable research interest in the link between 5-HT_{2C} receptors and addictive behaviors associated with food and psychostimulant drugs (Higgins and Fletcher, 2015; Howell and Cunningham, 2015). It is now clear that 5-HT_{2C} receptor agonists reduce palatable food consumption and other effects associated with obesity (increased body mass and fat content; Heisler et al., 2006, 2007a,b; Lam et al., 2008; Higgs et al., 2011, 2016). These agents can also disrupt various steps in the sequence of events leading up to compulsive use of addictive drugs such as cocaine and nicotine (including stimulant action, positive reinforcement, behavioral sensitization, and reinstatement). The mechanism is, in part, likely to involve a 5-HT_{2C} receptor–mediated decrease in mesolimbic dopamine function, but interactions with other transmitter systems (especially cortical glutamate) are likely to play a role. In addition, the lowering of food intake and metabolism by 5-HT_{2C} receptor agonists is likely to involve an action on hypothalamic nuclei that promotes satiety and regulates energy balance pathways (Heisler et al., 2006, 2007; Lam et al., 2008).

Interestingly, separate studies have revealed a link between 5-HT_{2C} receptors and impulse control; thus, 5-HT_{2C} receptor agonists reduce premature responses, whereas 5-HT_{2C} receptor antagonists have the opposite effect (Higgins et al., 2003; Winstanley et al., 2004; Fletcher et al., 2007). Given that impulsivity may influence many aspects of addictive behavior, 5-HT_{2C} receptors may modulate addiction indirectly via its effects on impulsive behavior.

Many of these experiments detect opposing interactions between 5-HT_{2C} receptors and 5-HT_{2A} receptors (e.g., 5-HT_{2A} receptor antagonists also lower impulsivity) that were detected in earlier neuropharmacological studies (Berendsen and Broekkamp, 1990). This opposing interaction between 5-HT_{2C} and 5-HT_{2A} receptors could explain the poor efficacy in drug addiction models of drugs that elevate 5-HT itself (SSRIs, fenfluramine). Consequently, it is proposed that an optimal way to control addictive behavior (and also avoid potential adverse effects associated with 5-HT_{2C} receptor agonists that have off-target effects at 5-HT_{2A} and 5-HT_{2B} receptors) may be a drug that combines in the same molecule agonist activity at the 5-HT_{2C} receptor and antagonist activity at the 5-HT_{2A} receptor (Anastasio et al., 2015; Higgins and Fletcher, 2015). Additional approaches for future investigation could include 5-HT_{2C} receptor agonists with biased agonist properties or positive allosteric modulators of the 5-HT_{2C} receptor.

4. 5-HT_3 Receptors. Outside of the well established role of central (as well as peripheral) 5-HT_3 receptors in the control of emesis, 5-HT_3 receptors have been linked to multiple behavioral effects, ranging from changes in anxiety and cognition to altered pain processing and sensitivity to addictive drugs. Most of the original evidence for this comes from reports of the behavioral effects of 5-HT_3 receptor ligands (mostly antagonists) in animal models; these and more recent findings are extensively reviewed elsewhere (Costall and Naylor, 1992; Bentley and Barnes, 1995; Barnes and Sharp, 1999; Walstab et al., 2010; Gupta et al., 2016). However, many of the behavioral effects observed in preclinical investigations are not confirmed by studies of selective 5-HT_3 receptor antagonists in clinical populations (Bentley and Barnes, 1995; Walstab et al., 2010). The apparent failure for the translation of these preclinical findings could, in part, be explained by suboptimal clinical dosing, as bell-shaped dose-response curves are often observed for 5-HT_3 receptor antagonists, particularly in relation to CNS effects. Nevertheless, the involvement of 5-HT_3 receptors in some of these behaviors receives support from more recent studies of mutant mice with altered 5-HT_{3A} receptor expression. In particular, support for a role for 5-HT_3 receptors in depression/anxiety-related behaviors, learning and memory, and pain processing comes from the phenotypic analysis of transgenic mice with 5-HT_{3A} receptor knockout or overexpression (Harrell and Allan, 2003; Kelley et al., 2003; Berger and Tecott, 2006). However, interpretation of the phenotypes of these mice is complicated by findings that 1) they are not always phenocopied by pharmacological agents, 2) they are dependent on the mouse background strain, and 3) overexpression and deletion of the 5-HT_{3A} receptor sometimes produced similar effects [for review, see Berger and Tecott (2006) and O'Leary and Cryan (2010)].

More recent evidence associating 5-HT_3 receptors with emotional behaviors and cognition comes from findings with vortioxetine, which has a complex pharmacology leading to potent inhibition of 5-HT_3 receptors, although the drug is also a 5-HT_7 and 5-HT_{1D} receptor antagonist, 5-HT_{1B} receptor partial agonist, 5-HT_{1A} receptor agonist, and 5-HT transporter inhibitor. Vortioxetine has significant antidepressant and procognitive activity in both rodent models and clinical trials (Mørk et al., 2012; Sanchez et al., 2015) and is currently marketed as an antidepressant with cognition-enhancing properties. Despite vortioxetine's polymodal pharmacology, inhibition of 5-HT_3 receptors is thought to play a prominent role in its mechanism of action. Thus, in rodents, vortioxetine preferentially occupies 5HT_3 receptors and the 5-HT transporter at low doses, and either 5-HT_3 receptor blockade alone produces similar effects to vortioxetine or effects of vortioxetine can be replicated by coadministration of an SSRI and a 5-HT_3 receptor antagonist (Mørk et al., 2012; Sanchez et al., 2015). One current mechanistic explanation of these findings is that blockade of 5-HT_3 receptors on specific populations of

GABA interneurons in the cerebral cortex contributes to vortioxetine's action (Riga et al., 2016).

5. 5-HT$_4$ Receptors. Early preclinical studies detected positive effects of 5-HT$_4$ receptor agonists on cognitive performance as well as a reduction in anxiety-related behaviors [for review, see Barnes and Sharp (1999)], and these observations have proven to be consistent in later work as recently reviewed by others (Bockaert et al., 2011; Claeysen et al., 2015; Hagena and Manahan-Vaughan, 2017). Stemming in part from studies of 5-HT$_4$ knockout mice (Compan et al., 2004), a role of 5-HT$_4$ receptors in feeding behavior also seems clear, with 5-HT$_4$ receptor agonists and antagonists having hypo- and hyperphagic properties, respectively (Jean et al., 2007; Bockaert et al., 2011).

Procognitive effects of 5-HT$_4$ receptor agonists have been described across a range of species and in a variety of experimental paradigms that model different aspects of short- and long-term memory, many of them dependent on the hippocampus where 5-HT$_4$ receptors are reasonably abundant (Hagena and Manahan-Vaughan, 2017). These agents also reverse the cognitive deficits induced by factors such as ageing, pharmacological interventions (e.g., muscarinic antagonists), and Alzheimer disease–like pathology. The mechanisms underlying the procognitive effects of 5-HT$_4$ receptor agonists are not certain but may be mediated by one or more of increased release of acetylcholine, induction of synaptic plasticity, increased synaptic spine formation, and altered hippocampal network properties [see Boddeke and Kalkman (1990), Claeysen et al. (2015), and Hagena and Manahan-Vaughan (2017)]. Administration of 5-HT$_4$ receptor agonists is also associated with increased amyloid precursor protein cleavage, which has led to speculation that these agents may be useful in the management of Alzheimer disease (Claeysen et al., 2015). A contribution from 5-HT$_4$ receptors in hippocampus seems likely as noted above, but a role for other circuits such as corticostriatal connectivity, which is abundant in 5-HT$_4$ receptors, seems likely.

Administration of 5-HT$_4$ receptor agonists is also associated with rapid antidepressant effects in animal models (Lucas et al., 2007), and there is preclinical evidence that 5-HT$_4$ receptor activation plays an important role in the action of SSRIs (Mendez-David et al., 2014). Thus, 5-HT$_4$ receptor agonists induce a number of responses in common with repeated antidepressant treatment (e.g., efficacy in models of depression or increased expression of neural plasticity markers) but with a rapid onset of action (Vidal et al., 2014b; Samuels et al., 2016). Moreover, antidepressant administration causes adaptive changes in 5-HT$_4$ receptor expression and function (Licht et al., 2010a). Part of the mechanism involved in the antidepressant effects of 5-HT$_4$ receptor agonists may be the activation of a positive feedback control of midbrain 5-HT neurons via 5-HT$_4$ receptor located in the frontal cortex (Lucas et al., 2005; Licht et al., 2010b).

The full interpretation of this literature on the behavioral effects of 5-HT$_4$ receptor ligands is somewhat hampered by the current lack of reports concerning the effect of these agents on mood or cognitive performance in humans, although there is early evidence that a 5-HT$_4$ receptor agonist improved the cognitive performance in nonhuman primates (Terry et al., 1998). Concerns about the potential for 5-HT$_4$ receptor agonists to elicit adverse gastrointestinal and cardiac effects are likely to have held back the transition to clinical studies, but these do not appear to be effects common to all 5-HT$_4$ receptor agonists (Claeysen et al., 2015), which is encouraging for future clinical studies.

6. 5-HT$_5$ Receptors. To date, there are few studies on the CNS effects of 5-HT$_5$ receptor ligands, and the main indications regarding the functions of this receptor currently come from studies on the phenotype of 5-HT$_{5A}$ receptor knockout mice (Grailhe et al., 1999). It is reported that these mice display increased exploratory activity in the open field and various other tests without evidence of altered anxiety levels. Without data from phenocopying experiments using 5-HT$_5$ receptor antagonists or other controls for developmental origins of the phenotype, it remains uncertain whether these behaviors are driven by the absence of 5-HT$_5$ receptor in the adult brain. Intriguingly, in 5-HT$_{5A}$ receptor knockout mice the locomotor activation induced by LSD was blunted, suggesting that 5-HT$_{5A}$ receptor activation might contribute to the psychotropic effects of psychedelic agents.

7. 5-HT$_6$ Receptors. Despite the identification of the 5-HT$_6$ receptor over 20 years ago, the functions of this receptor have been somewhat obscure until recently. Largely through studies on the effects of 5-HT$_6$ receptor ligands (agonists and antagonists) in animal behavioral models, it has been discovered that the receptor likely plays an important role in cognition.

The evidence for procognitive effects of 5-HT$_6$ receptor ligands is in keeping with the predominant localization of 5-HT$_6$ receptors in corticostriatal circuitry and is reviewed elsewhere (King et al., 2008; Codony et al., 2011; Meneses, 2015; see also *XIV. 5-HT$_6$ Receptors*). There are consistent findings that 5-HT$_6$ receptor antagonists enhance learning and memory mechanisms in a variety of preclinical models ranging from novel object and social recognition to spatial memory tasks. These effects are observed in naive animals and in those with memory deficits induced by, for example, pharmacological means such as cholinergic antagonists.

The mechanism underlying this effect of 5-HT$_6$ receptor blockade is uncertain. An interaction with the cholinergic system was proposed in early studies, and this is supported by more recent data. In particular, treatment with a 5-HT$_6$ receptor antagonist augments

the effects of an acetylcholinesterase inhibitor on cognitive measures in both animal models and patients with Alzheimer disease (Wilkinson et al., 2014; Kucinski et al., 2017; although 5-HT_6 receptor antagonists have failed in larger phase III clinical trial of patients with pathologic cognitive deficits), and there is neurophysiological and neurochemical evidence that this drug combination has a synergistic effect on cholinergic function (Herrik et al., 2016). However, recent data show that 5-HT_6 receptors are not expressed by cholinergic neurons (Helboe et al., 2015), which would invoke an indirect mechanism. A mechanism involving increased information processing through corticostriatal circuits was recently proposed (Kucinski et al., 2017).

A further complication in understanding the procognitive effects of 5-HT_6 receptor antagonists is the paradoxical observation that 5-HT_6 receptor agonists are also reported to have precognitive effects in some, albeit not all, studies (e.g., Schechter et al., 2008; Burnham et al., 2010; Kendall et al., 2011; Meneses et al., 2011). It is difficult to see how this paradox can be reconciled by inferring subtleties in subpopulations of 5-HT_6 receptor at the neural circuit level. Part of the explanation could lie in the cognitive domain being measured, and to date, few studies have systematically compared 5-HT_6 receptor agonists and antagonists in the same model. There also remains the intriguing possibility that the full answer lies in the pharmacology of 5-HT_6 receptor ligands and that their categorization in terms of partial agonist, inverse agonist, and even biased agonist is not yet complete (e.g., Romero et al., 2006).

8. 5-HT_7 Receptors. As with the 5-HT_6 receptor, the functions of the 5-HT_7 receptor are only now beginning to be revealed. This has largely come about through developments in 5-HT_7 receptor pharmacology and genetic mouse models. Preclinical data link the 5-HT_7 receptor to a variety of CNS processes, including regulation of circadian rhythms, body temperature, mood, cognitions, seizure threshold, and pain processing as well as mechanisms of addiction, and this is extensively reviewed elsewhere and will only be briefly discussed here (Hedlund, 2009; Hauser et al., 2015; see also *XV. 5-HT_7 Receptors*).

The link between the 5-HT_7 receptor and circadian rhythms was established in early studies, which recognized that the circadian phase shift of neurons of the suprachiasmatic nucleus evoked by the 5-HT_{1A} receptor agonist 8-OH-DPAT was mediated by 5-HT_7 and not 5-HT_{1A} receptors (Lovenberg et al., 1993). Indeed, in this light, re-evaluation of the hypothermic effect of 8-OH-DPAT using 5-HT_7 receptor knockout mice and selective antagonists revealed the involvement of 5-HT_7 receptors, although 5-HT_{1A} receptors appear to mediate the hypothermic effect of higher doses of the drug (Hedlund et al., 2004). This hypothermic effect of 5-HT_7 receptor activation is sufficiently robust to make it a useful test of 5-HT_7 receptor agonist activity in vivo (Di Pilato et al., 2014).

The discovery that 5-HT_7 receptor knockout mice have an antidepressant-like phenotype contributed to evidence that 5-HT_7 receptors have a role in emotional control. This feature of the mice is phenocopied by selective 5-HT_7 receptor antagonists that were shown to have antidepressant effects in a range of models in rats and mice. These agents also augment the effect of SSRI and other antidepressants in behavioral and neurochemical models. These findings have translated into clinical trials of the 5-HT_7 receptor antagonist JNJ-18038683 (Bonaventure et al., 2012), although a clear conclusion regarding efficacy of this agent has not yet been reached. It is interesting that in animal models, the antidepressant efficacy of amisulpiride is 5-HT_7 receptor–dependent. Many other antidepressant and antipsychotic drugs (tricyclics, lurasidone, aripiprazole, etc.) in clinical use have 5-HT_7 receptor antagonist properties, though the contribution of this receptor to their clinical effects is not known.

As a final point of interest, selective brain penetrant 5-HT_7 receptor agonists are becoming available as pharmacological tools (Di Pilato et al., 2014) to help further define the function of the 5-HT_7 receptor and open new therapeutic avenues.

XIX. 5-HT Receptors and the Cardiovascular System

A. Introduction

Cardiovascular effects of 5-HT are complex: when infused into the rat circulation, 5-HT produces a triphasic response with a short-lasting hypotensive phase, followed by a hypertensive phase and then a long-lasting hypotensive phase mediated by, respectively, 5-HT_3, 5-HT_2, and 5-HT_1 receptors (Kalkman et al., 1984). The cardiovascular effects of 5-HT have been known since the middle ages when ergots produced devastating peripheral vasoconstriction, which resulted in gangrene and limb loss because of consumption of rye bread polluted with fungi that produced ergots. For a long time, the general view was that 5-HT_2 receptors are largely responsible for vasoconstrictor effects, which would be blocked by 5-HT_2 receptor antagonists, such as ketanserin. However, the situation was much more complex, and it was known that depending on vessel and species, compounds such as the ergolines (ergotamine, DHE, and metergoline) would produce very complex responses: often vasoconstriction, but not infrequently vasodilation, could be observed as well. Even before the full repertoire of 5-HT receptors were known, it was suspected that "5-HT_1-like" receptors would at least mediate some of these effects. One of the most important tools for probing "5-HT_1-like receptor"–mediated effects was 5-carboxamidotryptamine

(5-CT), which became available in the early 1980s. 5-CT 1) potently contracted the dog saphenous vein (Feniuk et al., 1981); 2) inhibited the release of noradrenaline and 5-HT from sympathetic and central 5-HT neurons, respectively (Feniuk et al., 1981; Engel et al., 1983); and 3) displayed nanolomar affinity for the 5-HT_1 receptor recognition sites (Engel et al., 1983). Furthermore, several 5-CT–induced responses (associated with "5-HT_1–like" receptor recognition sites) were blocked by methiothepin and/or methysergide, but not by ketanserin, including relaxation of smooth muscle, contraction of dog saphenous vein, long-lasting hypotension in the rat, and tachycardia in the cat [see Kalkman et al. (1984), Bradley et al. (1986), Saxena and Villalón (1990)]. The migraine program led by Pat Humphrey at Glaxo used 5-CT as a template to study such effects with the idea to develop a new migraine medication that would be selective for the craniovascular bed (Humphrey et al., 1991). This research culminated with the discovery and development of sumatriptan (and other "triptans" that followed). It was then realized that sumatriptan had high affinity and potency at 5-HT_{1D} receptor sites before the actual receptors were cloned (Peroutka et al., 1989; Schoeffter and Hoyer, 1989a,b; Hoyer et al., 1990). The high affinity of sumatriptan for 5-HT_{1D} receptor recognition sites suggested that the sumatriptan-induced vasoconstriction was mediated by 5-HT_1–like receptors resembling the 5-HT_{1D} receptor subtype (Martin, 1994). However, the concept of 5-HT_1–like receptor remained alive for some time (Hoyer et al., 1994). One of the main reasons for this uncertainty was that metergoline, a potent 5-HT_{1D} receptor ligand, was less active as an antagonist both in vitro (e.g., canine, human, and rabbit saphenous vein and rabbit renal and cerebral arteries; Deckert et al., 1994; Hoyer et al., 1994) and in vivo (porcine and canine carotid vascular beds; Den Boer et al., 1992; Villalón et al., 1995) than methiothepin, as may have been anticipated from metergoline's affinity at the then defined 5-HT_{1D} receptor recognition sites. However, the experimental conditions used to detect 5-HT_{1D} receptor binding (Waeber et al., 1988) allowed binding to, at the time unknown, 5-ht_{1e}, 5-HT_{1F}, and 5-HT_7 receptors, which clearly complicated interpretation. The cloning of 5-$HT_{1D\alpha}$ and 5-$HT_{1D\beta}$ receptors combined with their relatively high affinity for sumatriptan, and the eventual distinction between 5-HT_{1B} and 5-HT_{1D} receptors, including the species differences, allowed some order to the complexity (Weinshank et al., 1992) along with an appreciation of a need for subtype-selective 5-HT_1 receptor agonists and antagonists. Eventually it was realized that the loosely defined "5-HT_1–like receptor" covered at least three structurally distinct receptors, namely, the 5-HT_{1B}, 5-HT_{1D}, and 5-HT_7 receptors. Here, basic and more recent knowledge as to the involvement of each 5-HT receptor in the cardiovascular system in normal physiological and pathophysiological conditions will be discussed (the cardiovascular system to include the heart, blood, vasculature, kidneys, and adrenals as well as the peripheral and central nervous systems involved in cardiovascular system regulation).

B. 5-HT in Cardiovascular Tissues

The majority of 5-HT in the body is made by 1) the gastrointestinal system and 2) the neurons of the raphe complexes in the brainstem. 5-HT makes its way to all body organs by storage in blood platelets following SERT uptake and through free 5-HT being released from the enterochromaffin cells of the intestine. Free 5-HT—external to the platelet—is measurable in the plasma of all species examined (Watts et al., 2012). 5-HT has been detected in all rat tissues examined (Linder et al., 2009) and cannot be accounted for by the presence of platelets given that electron microscopic images show no platelet adhesion to the tissues used for measuring 5-HT. Moreover, 5-hydroxyindole acetic acid (5-HIAA) can be detected in these same tissues. 5-HIAA is a metabolite of 5-HT, the product of monoamine oxidase, a mitochondrial enzyme (i.e., 5-HT has to be inside the cell to be converted to 5-HIAA). Interestingly, many cardiovascular tissues express SERT, and, at least in the rat, SERT-dependent uptake of 5-HT takes place in peripheral tissues that include arteries, heart, and adrenals (Linder et al., 2009). The SERT KO rat, created by Edwin Cuppen (Homberg et al., 2007), has been particularly useful in these studies. Moreover, certain tissues of the cardiovascular system synthesize 5-HT, independent of the gastrointestinal system and brain, using tryptophan hydroxyalase (TPH), of which two forms exist, TPH1 and TPH2, the latter being neuron-specific. This includes vascular smooth muscle (Ni et al., 2004, 2008), endothelial cells (Morecroft et al., 2007), kidney (Hafdi et al., 1996; Sole et al., 1986; Stier and Itskovitz, 1985; Stier et al, 1985), cardiomyocytes (Ikeda et al., 2005; Pönicke et al., 2012) and potentially adrenal glands (Brownfield et al., 1985; Csaba and Sudar, 1978; Delarue et al., 1992; Holzwarth and Brownfield, 1985; Holzwarth et al., 1984; Pönicke et al., 2012; Verhofstad and Jonsson, 1983).

C. Cardiovascular Effects Mediated by 5-HT Receptors

1. 5-HT_{1A} Receptors. One of the best-known peripheral actions of 5-HT_{1A} receptor stimulation in rodents is inhibition of stress-evoked cardiovascular responses, reducing the tachycardia and renal sympathoexcitation that accompany stress (Horiuchi et al., 2005, 2011; Nalivaiko and Sgoifo, 2009). Stress models investigated include restraint stress, fear-conditioned stress, cold exposure, and elevated plus maze (anxiety-producing). Interestingly, 5-HT_{1A} receptor agonists can be given systemically to produce their anxiolytic effects independent of sympathoinhibition (Vianna and Carrive, 2009). 5-HT_{1A} receptors also mediate increased vagal drive (Ramage, 1990).

5-HT_{1A} receptor agonists lower blood pressure in the normal (nonhemorrhaged, nonstressed) rat. 8-OH-DPAT given in the ventral medulla of the normal rat causes hypotension and bradycardia (Helke et al.,

1993). Similarly, 8-OH-DPAT, flesinoxan, and other 5-HT_{1A} receptor agonists reduce blood pressure in both the spontaneously hypertensive rat and Wistar Kyoto rat (see, e.g., Doods et al., 1988). The 5-HT_{1A} receptor agonist flesinoxan has been suggested to inhibit sympathetic nerve activity, at least in part, through renal nerves and to lower blood pressure in rats (Chamienia and Johns, 1994) and in cats (Wouters et al., 1988; Ramage et al., 1992). Collectively, these studies point to the central role of 5-HT_{1A} receptors modifying autonomic responses as one of the greatest contributions made by this receptor subtype. Interestingly, peripheral 5-HT_{1A} receptors can also mediate inhibition of the sympathetic vasopressor outflow in pithed rats (Villalón et al., 1998; Morán et al., 1998).

In contrast to the decrease in blood pressure caused in a normal rat, 8-OH-DPAT increases whole-body venous tone to protect against hemorrhagic shock (Tiniakov and Scrogin, 2009), described as a sympathetic medicated venoconstriction. 5-HT_{1A} receptors are not highly expressed in the vasculature, and direct effects within the vasculature are uncommon (Villalón and Centurión, 2007; Ramage and Villalón, 2008). Flesinoxan and other 5-HT_{1A} receptor agonists have been developed as blood pressure–lowering agents, but no clinical success has been reported, and these programs were abandoned.

2. 5-HT_{1B} Receptors. 5-HT_{1B} receptors are found on cerebral arteries and other vascular tissues mediating direct vasoconstriction [see Villalón et al. (2003) and Villalón and Centurión (2007)]. Furthermore, it seems that the receptor may be silent and may become responsive in conditions such as atherosclerosis (Geerts et al., 2000; Ishida et al., 2001). Peripheral effects in rats have been described, such as 1) inhibition of noradrenaline release from sympathetic nerves in vena cava (Göthert et al., 1986) and systemic vasculature (Villalón et al., 1998) and 2) inhibition of plasma extravasation produced by trigeminal ganglion stimulation (Buzzi and Moskowitz, 1991). 5-HT_{1B} receptors also mediate vasoconstriction in the rat caudal arteries (Craig and Martin, 1993) and the canine external carotid circulation (De Vries et al., 1998).

5-HT_{1B} receptors are expressed in vascular smooth muscle of several different arteries, including rat aorta (Banes and Watts, 2003), aortic vasavasorum (Cohen et al., 2002), tail artery (Craig and Martin, 1993), and middle cerebral artery (Kovács et al., 2012); human arteries and veins, including central, pulmonary, and coronary arteries (Verheggen et al., 1998, 2004; Morecroft et al., 1999; Nilsson et al., 1999a,b; van den Broek et al., 2002; Tanaka et al., 2008b) as well as coronary endothelial cells (Ishida et al., 1998); canine internal (Centurión et al., 2001) and external (-Centurión et al., 2001, Valdivia et al., 2004) carotid artery beds; rabbit saphenous vein, basilar artery (Bhattacharya et al., 2004), and renal artery (Hill et al., 2000); porcine coronary artery (Schoeffter and Hoyer, 1989, 1990); bovine pulmonary artery (McKenzie et al., 2010); and guinea pig isolated iliac artery (Sahin Erdemli et al., 1991; Jahnichen et al., 2004). Next to the 5-HT_{2A} receptor, the 5-HT_{1B} receptor is the best studied and most frequently reported in the vasculature. Expression in cerebral arteries has been a significant focus given the potential involvement of these receptors in the pathophysiology of migraine (e.g., Villalón et al., 2003; Villalón and Centurión, 2007).

The 5-HT_{1B} receptor mediates vasoconstriction in a variety of blood vessels, although some 5-HT_{1B} receptors have been described as "silent," meaning that a vessel needs to be primed with a depolarizing stimulus such as high K^+ before a functional receptor is observed ("unmasking") (Movahedi and Purdy, 1998; Froldi et al., 2008). By contrast, functional 5-HT_{1B} receptors, coupled to nitric oxide synthase, have been reported in cultured bovine aortic endothelial cells (McDuffie et al., 1999). There is thus the potential for 5-HT_{1B} receptors to serve opposing actions in the blood vessel: contraction through smooth muscle and relaxation through the endothelium (Schoeffter and Hoyer, 1989, 1990; Sahin Erdemli et al., 1991).

Functional 5-HT_{1B} receptors are also found in the trigeminocervical complex of cats and dogs, where activation of the receptor inhibits 1) the "nociceptive traffic" within this complex in the former (Goadsby and Classey, 2003) and 2) capsaicin-sensitive trigeminal sensory nerves innervating the external carotid bed in the latter (Muñoz-Islas et al., 2006, 2009). 5-HT_{1B} receptor agonists inhibit the release of sensory neuropeptides (particularly calcitonin gene–related peptide) in migraine, providing the basis for an effective treatment in addition to the craniovascular vasoconstrictor effects (Ma et al., 2001; Villalón and Olesen, 2009; Gupta and Villalón, 2010). Finally, prejunctional 5-HT_{1B} receptors inhibit (by peripheral mechanisms) 1) norepinephrine release in blood vessels (Molderings et al., 1990, 1996; Villalón et al., 2001), 2) the vasopressor (Villalón et al., 1998) and cardioaccelerator (Sánchez-López et al., 2004) sympathetic outflow, and 3) the vasodepressor sensory CGRPergic outflow in rats (González-Hernández et al., 2011). Thus, the expression and function of 5-HT_{1B} receptors is complex and at multiple levels, even within just the vasculature.

In the vasculature, the 5-HT_{1B} receptor is best known for its upregulation and elevated function in pulmonary hypertension (Keegan et al., 2001). The 5-HT_{1B} receptor contractile function is also enhanced in rabbit atherosclerotic coronary arteries (Ishida et al., 2001) and rabbit carotid arteries from animals subjected to a carotid collar (Geerts et al., 2000). Also well established is the use of 5-$HT_{1B/1D/1F}$ receptor agonists (the triptans) for the abortive treatment of migraine attacks (e.g., Villalón et al., 2003). Though useful in causing a cerebral vasoconstriction that is thought to be associated with improved symptoms, the triptans can also cause a coronary artery constriction that limits their use in the treatment of migraine in patients with a cardiac

condition; however, long-term surveillance shows a remarkably low incidence of serious side effects (Chan et al., 2011). Exploration for antimigraine mechanisms have expanded beyond the 5-HT_{1B} receptor (there are a multitude of triptans available) with interest on the role of the 5-HT_{1F} receptor playing a potentially nonvascular role (Chan et al., 2011; Rizzoli, 2014) with compounds such as lasmiditan.

3. 5-HT_{1D} Receptors. The external carotid vasoconstrictor responses to 5-HT and sumatriptan are antagonized by the selective 5-HT_{1B} receptor antagonist SB224289 but not the selective 5-HT_{1D} receptor antagonist BRL15572 (De Vries et al., 1998), indicating that 5-HT_{1B} receptors mediate this vasoconstrictor response. It must be noted that sumatriptan and second-generation triptans do not distinguish between 5-HT_{1B} and 5-HT_{1D} receptors (Villalón et al., 2003; Villalón and Centurión, 2007).

A series of isochroman-6-carboxamide derivatives, including PNU-109291 and PNU-142633, have been described as highly selective 5-HT_{1D} receptor agonists (Ennis et al., 1998; McCall et al., 2002). These 5-HT_{1D} receptor agonists do not produce vasoconstriction in in vivo (Centurión et al., 2001) or in vitro preparations (cerebral arteries; Bouchelet et al., 2000).

At the peripheral level, the presence of 5-HT_{1D} receptors seems to be rather limited to autonomic and trigeminal nerve terminals/ganglia (Jones et al., 1995; Molderings et al., 1996; Shepheard et al., 1997; Villalón et al., 1998).

There were attempts to develop 5-HT_{1D} receptor agonists for migraine therapy [for references, see Villalón et al. (2003) and Chan et al. (2011)]; these compounds are active in the trigeminal inflammation/plasma extravasation models yet would presumably be less prone to side effects, as the cardiovascular effects are largely negligible (Villalón et al., 2003; Chan et al., 2011). However, when tested in the clinic to treat migraine, there was little success, and development has been abandoned.

4. 5-ht_{1e} Receptors. The receptor has been immunohistochemically localized to the cerebral vasculature of humans, but mice and rats do not express a functional 5-ht_{1e} receptor (Klein and Teitler, 2012). There is no evidence for a physiologic role for 5-ht_{1e} receptors (Hoyer et al., 1994; Hoyer and Martin, 1997; Villalón and Centurión, 2007; Ramage and Villalón, 2008).

5. 5-HT_{1F} Receptors. 5-HT_{1F} receptor mRNA and the corresponding protein is preferentially expressed in neuronal tissues rather than vascular smooth muscle (Ullmer et al., 1995; Bouchelet et al., 1996). Accordingly, 5-HT_{1F} receptor agonists appear devoid of direct vasoconstrictor properties (Johnson et al., 1998; Cohen et al., 1999; Villalón et al., 1999).

Most recently, lasmiditan was developed as a 5-HT_{1F} receptor agonist (Rizzoli, 2014); it did not contract rabbit saphenous vein, which is frequently used as a surrogate for the human coronary artery for which crossover effects of migraine drugs (triptans) can be identified (Nelson et al., 2010). This is consistent with a lack of contractile response via the 5-HT_{1F} receptor generally in this preparation (Cohen and Schenck, 2000) as well as with human cerebral and meningeal arteries (Razzaque et al., 1999; Bouchelet et al., 2000).

6. 5-HT_{2A} Receptors. The 5-HT_{2A} receptor is the original "D" receptor of Gaddum and Picarreli (1957). It is expressed in vascular smooth muscle, cardiac muscle, and platelets in multiple species, including humans (Ullmer et al., 1995; Derangeon et al., 2010; Watts et al., 2012).

The 5-HT_{2A} receptor mediates contraction in arteries and veins from most species (Villalón and Centurión, 2007), including rat (Sung et al., 2013), both directly and indirectly through regulation of sympathetic nerves (Blessing and Seaman, 2003). It promotes platelet aggregation and gap junctional coupling in heart myocytes (Derangeon et al., 2010), induces the activation of cardiac fibroblasts (Yabanoglu et al., 2009), and, within the nucleus tractus solitarius, stimulates a depressor and bradycardic response (Comet et al., 2007). Moreover, the direct component of 5-HT–induced tachycardia in reserpinized pithed rats is mediated by activation of 5-HT_{2A} receptors (Centurión et al., 2002). The receptor mediates increases in contractility in rat cardiac atrium (Läer et al., 1998) and ventricle but the latter only in heart failure and cardiac hypertrophy (Läer et al., 1998; Qvigstad et al., 2005c; Birkeland et al., 2007b; Brattelid et al., 2007a,b).

In most vascular diseases, isolated blood vessels are hyper-responsive to 5-HT via the 5-HT_{2A} receptor. Similar to the 5-HT_{1B} receptor, upregulation of the 5-HT_{2A} receptor has been implicated in elevated vascular tone in pulmonary hypertension (Delaney et al., 2013). The 5-HT_{2A} receptor antagonist sarpogrelate blocked the development of pulmonary hypertension induced by monocrotaline and increased survival rate for this highly fatal disease (Hironaka et al., 2003).

The 5-HT_{2A} receptor has been targeted for the treatment of hypertension; thus, ketanserin had been developed for this indication, but further research revealed that the antihypertensive effects of ketanserin were actually mediated by α_1-adrenoceptor blockade. Interestingly, relatively selective 5-HT_{2A} receptor antagonists (e.g., ritanserin) are devoid of antihypertensive potential in humans.

7. 5-HT_{2B} Receptors. The 5-HT_{2B} receptor, originally described in the stomach fundus, is expressed throughout the cardiovascular system, including vascular smooth muscle (many beds), endothelial cells, cardiac myocytes, fibroblast, and valves (Choi and Maroteaux, 1996; Jaffré et a., 2009).

In systemic arteries from normotensive rats, the 5-HT_{2B} receptor is expressed but is apparently not functional (Banes and Watts, 2002, 2003). The endothelial 5-HT_{2B} receptor mediates a nitric oxide–mediated relaxation in normal vessels (Ellis et al., 1995; Glusa and

Pertz, 2000; Jahnichen et al., 2005). Strong support for the interaction of the 5-HT_{2B} receptor with NOS was provided by Manivet et al., (2000). In endothelial cells isolated from human coronary artery, 5-HT elevates nitric oxide production through the 5-HT_{2B} receptor (Ishida et al., 1998). The receptor also mediates calcium release in human pulmonary arterial endothelial cells (Ullmer et al., 1996). The 5-HT_{2B} receptor would appear necessary for cardiomyocyte survival (Nebigil et al., 2003a,b); receptor ablation leads to a cardiomyopathy without hypertrophy (Nebigil et al., 2000, 2001).

The 5-HT_{2B} receptor is critical to development of hypoxia-induced pulmonary hypertension (Launay et al., 2002; Esteve et al., 2007) and monocrotaline-induced pulmonary hypertension (Zopf et al., 2011) and becomes functional in a number of models of experimental hypertension (Watts et al., 1995, 1996; Watts and Fink, 1999; Banes and Watts, 2002, 2003; Russell et al., 2002). Inhibition of the 5-HT_{2B} receptor appears to block valvular myofibroblast differentiation, a process involved in calcification of the aortic valves (Hutcheson et al., 2012). The question remains whether this role of the 5-HT_{2B} receptor also applies to pulmonary hypertension in humans, in which the 5-HT_{1B} receptor seems to have a central role (Maclean and Dempsie, 2010). However, the use of the anorectic combination fenfluramine-phentermine precipitated pulmonary hypertension and valvular heart disease, with evidence of mediation via the 5-HT_{2B} receptor (Fitzgerald et al., 2000; Rothman et al., 2000). Valvulopathy is also observed with other 5-HT_{2B} receptor agonists (e.g., norfenfluramine, benfluorex, pergolide, cabergoline, and ergotamine, most of which have been withdrawn from a majority of markets because of concerns around these adverse side-effects). As a consequence of these findings, preclinical safety screens for 5-HT_{2B} receptor agonism have become mandatory in an attempt to avoid the potential risk of valvulopathy (Hutcheson et al., 2011; Reid et al., 2013). Valvulopathy induced by serotonergic compounds did not begin with fenfluramine-phentermine; it has been observed with ergots over the centuries in central and eastern Europe and even in ancient Egypt (Hauck et al., 1990; Eadie, 2003) as well as in patients with carcinoid tumors (Druce et al., 2009). Finally, 5-HT_{2B} receptors appear essential for isoproterenol-induced cardiac hypertrophy in the mouse (Jaffre et al., 2004), and overexpression of the 5-HT_{2B} receptor led to cardiac hypertrophy in the mouse (Nebigil et al., 2003b). It should also be kept in mind that MDMA (ecstasy) and its metabolite MDA, as well as a number of MDMA analogs, act as 5-HT_{2B} receptor agonists and thus carry the risk of valvulopathies if consumed (Setola et al., 2003).

8. 5-HT_{2C} Receptors. This receptor has not been localized to peripheral tissues with confidence, though a few reports suggest this may be so. The 5-HT_{2C} receptor is, however, expressed in cardiovascular-controlling areas of the central nervous system (e.g., nucleus tractus solitarius) and may elevate blood pressure (Ferreira et al., 2005; Austgen et al., 2012).

9. 5-HT_{3A} and 5-HT_{3AB} Receptors. An important action of 5-HT_3 receptors in the cardiovascular system is the ability to elicit the neuronally mediated transient von Bezold-Jarisch reflex [for references, see Kalkman et al. (1984), Saxena and Villalón (1990), and Villalón and Centurión (2007)].

Alternatively, activation of some peripheral 5-HT_3 receptors evokes tachycardic responses that may involve 1) noradrenaline release from postganglionic cardiac sympathetic neurons (Saxena and Villalón, 1990), 2) a direct action on the cardiac pacemaker (Wilson et al., 1990), and 3) CGRP release from cardiac sensory nerves (Nishio et al., 2002).

5-HT_3 receptors in sympathetic ganglia may sustain chronic stress–induced hypertension in the rat (Nalivaiko and Sgoifo, 2009).

10. 5-HT_4 Receptors. Cardiac expression is well established for the 5-HT_4 receptor, with earliest papers of 5-HT_4–like receptors in human atria dating back to 1989 (Kaumann and Levy, 2006). The 5-HT_4 receptor is functional in fetal hearts (Brattelid et al., 2012), normally expressed in human and porcine atrium and ventricle (Bach et al., 2001; Brattelid et al., 2004a,b; Weninger et al., 2012), and splice variants of the receptor exist in the human heart (Bach et al., 2001; Brattelid et al., 2004a; Krobert et al., 2005; Kaumann and Levy, 2006). Endothelial cells also express 5-HT_4 receptors (Nishikawa et al., 2010; Machida et al., 2013), as does the adrenal gland (Vilaró et al., 2002).

5-HT_4 receptors mediate positive inotropic, chronotropic, and lusitropic effects in human and porcine atrium (Kaumann, 1990; Kaumann et al., 1990, 1993; Villalón et al., 1990, 1991; Krobert et al., 2005; De Maeyer et al., 2006; Kaumann and Levy, 2006; Gergs et al., 2009; Chai et al., 2012; Weninger et al., 2012) and positive inotropic and lusitropic effects in human and porcine ventricular myocardium (Brattelid et al., 2004b; Afzal et al., 2008).

5-HT_4 receptors also mediate arrhythmogenesis in human atria (Kaumann, 1994; Pino et al., 1998), modulation of angiogenesis in cultured human umbilical vein endothelial cells (Nishikawa et al., 2010; Profirovic et al., 2013), and aldosterone secretion from the adrenal gland (Lefebvre et al., 1998, 2000). Interestingly, a model of cardiac overexpression of the human 5-HT_4 receptor in the mouse heart has been developed to test for arrhythmogenesis as a cardiac side effect (Gergs et al., 2010).

As pointed out earlier, only 5-HT_{2A} receptors mediate 5-HT–induced cardiostimulation in healthy rats (Centurión et al., 2002), but both 5-HT_4 and 5-HT_{2A} receptors mediate this effect after development of congestive heart failure (Qvigstad et al., 2005a,c). Accordingly, 5-HT_4 receptors 1) become functional in ventricles during heart failure (Brattelid et al., 2004b, 2007a, 2012; Qvigstad et al., 2005a,c; Birkeland et al., 2007b) and 2) may contribute to human atrial

fibrillation (Kaumann et al., 1994; Lezoualc'h et al., 2007). Thus, 5-HT_4 receptor antagonists may have potential therapeutic usefulness for improvement of cardiac function. In line with a proposed arrhythmogenic effect of stimulation of atrial 5-HT_4 receptors (Kaumann, 1994), antiarrhythmic effects of a 5-HT_4 antagonist were demonstrated in a porcine model of atrial fibrillation (Rahme et al., 1999). However, although atrial 5-HT_4 expression levels increase in human chronic atrial fibrillation (Lezoualc'h et al., 2007), the arrhythmic potential of 5-HT_4 receptors may be reduced in established human atrial fibrillation (Christ et al., 2014).

By analogy with the use of betablockers to improve prognosis in heart failure (Lohse et al., 2003), the use of 5-HT_4 receptor antagonists was proposed (Qvigstad et al., 2005b; Levy et al., 2008) and delivered improvement in a rat model (Birkeland et al., 2007a). 5-HT_4 receptors are functional in human failing ventricle (Brattelid et al., 2004b; Afzal et al., 2008), and apparent clinical benefits of 5-HT_4 receptor antagonism have been detected (Kjekshus et al., 2009).

11. 5-HT_5 Receptors. The knowledge about cardiovascular responses mediated by 5-HT_5 receptors is very limited [see Ramage and Villalón (2008)]. It is suggested that putative 5-HT_5 receptors can mediate 1) the 5-HT–induced cardiac sympathoinhibition (together with 5-$HT_{1B/1D}$) receptors in pithed rats (Sánchez-López et al., 2003) and 2) the GR-127935–sensitive mechanism mediating hypotension in anesthetized rats (Sánchez-Maldonado et al., 2015).

12. 5-HT_6 Receptors. These receptors have not been localized or found to be functionally relevant within the cardiovascular system (Villalón and Centurión, 2007; Ramage and Villalón, 2008).

13. 5-HT_7 Receptors. The 5-HT_7 receptor most probably is encompassed with the originally designated "5-HT_1–like" receptor that mediates direct (endothelium-independent) vasorelaxation, the late vasodepressor response (i.e., the tertiary component of the triphasic response) following intravenous administration of 5-HT (Kalkman et al., 1984; Saxena and Villalón, 1990, 1991).

5-HT_7 receptors are primarily located in vascular smooth muscle of most species (Ullmer et al., 1995; Villalón and Centurión, 2007; Ramage and Villalón, 2008). The 5-HT_7 receptor mediates direct vasorelaxation in multiple vascular beds (e.g., Cushing et al., 1996; Terrón, 1996; Villalón et al., 1997a, 2001; Jahnichen et al., 2005; Seto et al., 2009; Watts et al., 2015).

14. Receptor-Independent Actions of 5-HT. It is recognized that 5-HT can exert a (patho)physiologic role that is independent of cell surface 5-HT receptors.

For instance, various groups have reported that animals lacking SERT, or those in which SERT was pharmacologically inhibited, are protected from pulmonary hypertension (Fanburg and Lee, 2000; Marcos et al., 2003; Guignabert et al., 2005; Elangbam et al., 2008; Wang et al., 2012). Similarly, rodents overexpressing SERT develop pulmonary hypertension (Guignabert et al., 2006). It is known that intracellular 5-HT is able to modify proteins, a phenomenon known as "serotonylation" of proteins (Lin et al., 2014). Such protein modification may mediate mitogenic and profibrotic effects of 5-HT independent of receptor activation (Guilluy et al., 2009; Liu et al., 2011; Wei et al., 2012; Penumatsa and Fanburg, 2014). This covalent modification of target proteins by 5-HT is mediated by the enzyme transglutaminase, of which the tissue transglutaminase isoform (TG II, tTG) is abundant in vascular tissue. Targets for serotonylation include Akt (Penumatsa et al., 2014); small GTPases such as Ras, Rab 4, and Rho (Ahmed et al., 2008; Mercado et al., 2011; Walther et al., 2003b, 2011; Lin et al., 2013); fibronectin (Liu et al., 2011; Hummerich et al., 2012); and smooth muscle α-actin (Watts et al., 2009).

XX. 5-HT Receptors and the Gastrointestinal Tract

A. Introduction

The GI tracts of mammals contain a huge store of 5-HT (Gershon and Tack, 2007), melatonin (a derivative of 5-HT; Stone and Darlington, 2002), and kynurenine derivatives of tryptophan, which can interact with 5-HT receptors on GI muscle (Pomfret et al., 1987). This 5-HT is released to act within the GI tract (all known 5-HT receptors are expressed within the GI tract; see below) and externally into the blood, playing various roles in metabolism, osteogenesis, immunity, neurogenesis, and neuroprotection (Gershon, 2012, 2013). Indeed, about 95% of the 5-HT in the human body the GI tract, with 90% being in enterochromaffin (EC) cells in the mucosal epithelium and 5% in the neural structures intrinsic to the bowel wall; in common with the rest of the epithelium, EC cells are continually shed and replaced. Large amounts of 5-HT are also present in mast cells of rats and mice, but human intestinal mast cells usually contain no 5-HT (Buhner and Schemann, 2012); a subset of mast cells in patients with colon carcinoma and ulcerative colitis has been reported to contain 5-HT (Stoyanova and Gulubova, 2002). In the human GI tract, the highest amounts of 5-HT are present in the duodenum and rectum, whereas the lowest is found in the esophagus and ileum (Spiller, 2008a). In rats, the greatest amount of 5-HT is in the cecum (Hansen and Witte, 2008). 5-HT is also present in a limited (2% to 3%) number of myenteric descending interneurons (with variation among species; Gershon, 2003; Gershon and Tack, 2007), potentially regulating secretion and circular muscle movements in guinea pig jejunum (Neal and Bornstein, 2007), and in enteric sensory and motor neurons of mouse colon (Okamoto et al., 2014).

There are two GI 5-HT pools, each dependent on a different isoform of TPH (Li et al., 2011b). The larger pool is present in EC cells and is TPH1-dependent. The smaller pool, present in neurons, is TPH2-dependent, as is CNS 5-HT. These two pools can thus be selectively

depleted. Thus, TPH1 KO depletes EC cells, whereas TPH2 KO depletes neuronal 5-HT.

5-HT is released from EC cells by mechanical and chemical stimuli (e.g., pH, bile acids, and nutrients such as glucose and short-chain fatty acids) applied to the luminal surface of the bowel. 5-HT release is modulated by neuronal and hormonal inputs acting at numerous receptors (including 5-HT receptors; see below) expressed by EC cells (Gershon, 2003; Hansen and Witte, 2008). The junctions between EC cells and intrinsic and extrinsic sensory nerve terminals in the mucosa are not morphologically like traditional synapses (Wade and Westfall, 1985). Indeed, 5-HT secreted by EC cells acts locally in a paracrine fashion, although the nerves it activates may be situated far from the EC cells (Wade and Westfall, 1985). In addition, EC cells are in constant motion from the crypts to the surface; they turn over and are replaced by stem cells both in the small and large intestines. As a result of the movement and transient nature of EC cells, traditional synapses are not found (nerves are not good at innervating moving targets) and EC cells do not focus the 5-HT released as precisely as do neurons [see Gershon and Tack (2007)]. Similarly, EC cells are located in relatively close proximity to mucosal lymphocytes (Yang and Lackner, 2004).

The 5-HT released into the mucosa and/or lumen does not normally penetrate to the muscle of the GI tract because of two reasons: first, 5-HT has poor ability to cross lipid layers of cell membranes, and second, 5-HT is readily taken up by neurons and enterocytes via either SERT or organic cation transporters (Wade et al., 1996; Chen et al., 1998). 5-HT is also removed by the vasculature where it is taken up into blood platelets, which lack TPH and thus cannot synthesize 5-HT. Mucosal 5-HT thus does not gain access to the muscle layers of the bowel, except in pathologic conditions in which high plasma concentrations are reached (Sanger, 2008).

The released 5-HT can potentially act at multiple 5-HT receptors [see Costa et al. (2003), Beattie and Smith (2008), Hansen and Witte (2008), Sanger (2008), Chetty et al. (2009), Li et al. (2011b), Hoffman et al. (2012), and Alexander et al. (2015a,c)], which are expressed by several elements of the GI tract:

- Isolated enteric crest-derived cells (mRNA for all known 5-HT receptors)
- EC cells (5-HT_{2C}, 5-HT_3, 5-HT_4)
- Goblet cells (5-HT_4)
- Enterocytes (5-HT_{1A}, 5-HT_{2A}, 5-HT_3, 5-HT_4)
- Muscle (5-$HT_{1B/1D}$, 5-HT_{2A}, 5-HT_{2B}, 5-HT_4, 5-HT_7)
- Interstitial cells of Cajal (5-HT_3, 5-HT_4)
- Motor (5-HT_{1A}, 5-$HT_{1B/1D}$, 5-HT_{2A}, 5-HT_{2B}, 5-HT_3, 5-HT_4) and sensory (5-HT_3, 5-HT_4, 5-HT_7) neurons of the myenteric and submucosal plexus
- Terminals of GI extrinsic sympathetic and/or parasympathetic nerves (5-HT_{1A}, 5-HT_3, 5-HT_4 in rodents)

The genes encoding the 5-HT_{3A} and 5-HT_{3B} subunits of the 5-HT_3 receptor are widely expressed by different mammalian tissues. In humans, expression of 5-HT_{3C} and 5-HT_{3E} mRNA is greatest within the GI tract (5-HT_{3C} is also present elsewhere), whereas 5-HT3D mRNA is largely restricted to the kidney, colon, and liver (Niesler et al., 2003; Holbrook et al., 2009; Yaakob et al., 2015). Notably, genes encoding 5-HT_{3C}, 5-HT_{3D}, and 5-HT_{3E} are found in humans and other mammals but not in rodents (Holbrook et al., 2009).

5-HT3A mRNA has been found in the mucosa and circular and longitudinal (*taenia*) muscle of human colon, whereas 5-HT_{3B} mRNA may be more common in muscle and 5-HT_{3E} mRNA in the mucosa (Chetty et al., 2009; Yaakob et al., 2015). Importantly, 5-HT_{3A} and 5-HT_{3B} mRNA and immunoreactivity are coexpressed with neuronal cell markers in the submucosal plexus of human intestine and therefore potentially form heteromeric 5-HT_{3AB} receptors in these nerves (Michel et al., 2005). Others report that in cell bodies of human colon myenteric neurons, 5-HT_{3C}, 5-HT_{3D}, and 5-HT_{3E} mRNA coexpress with 5-HT_{3A}, whereas mRNA for 5-HT_{3A} and 5-HT_{3D} coexpress in the submucosal plexus (Kapeller et al., 2011). The physiologic significance of these different expression patterns is not clear. However, the absence of genes encoding 5-HT_{3C}, 5-HT_{3D}, and 5-HT_{3E} in rodents has been linked to the specific divergence of rodents away from the primate evolutionary line and the peculiar lack of an emetic reflex in rodents (a function strongly linked in humans with 5-HT_3 receptors; see below and Holbrook et al., 2009). Here it may be of interest to note that selective 5-HT_3 receptor antagonists do not necessarily affect GI functions in the same way (e.g., Banner and Sanger, 1995), but whether such anomalies are explained by an ability to distinguish between different 5-HT_3 receptor subunits [see Thompson and Lummis (2013) for examples] is still unknown.

Among the 5-HT GPCRs, splice variants exist in human colon for 5-HT_{2B}, 5-HT_7, and 5-HT_4 receptors (Coupar et al., 2007; Chetty et al., 2009; Yaakob et al., 2015), although their roles are poorly understood. It has been speculated that the efficiency of 5-HT_4 receptor intracellular coupling and/or the desensitization liability and affinity of this receptor for particular ligands depends on which COOH-terminal splice variant is expressed by a particular cell (Coupar et al., 2007; Beattie and Smith, 2008; Sanger, 2009). This would explain why 5-HT_4 receptor agonists facilitate GI cholinergic functions with high intrinsic activity (leading to increased GI motility), whereas in cardiac muscle and other tissues, the intrinsic activity of the same agonist is low (De Maeyer et al., 2006). Notably, the 5-$HT_{4(d)}$ isoform is specifically expressed within human intestine (in addition to 5-$HT_{4(b)}$ and 5-$HT_{4(a)}$, which are widely expressed), and the GI prokinetic renzapride (a nonselective 5-HT_4 receptor agonist; Sanger, 1987a)

acts as a full agonist at this isoform but only as a partial agonist at the 5-$HT_{4(g)}$ isoform (Mialet et al., 2000).

Certain 5-HT GPCRs may modulate ion channel functions within the GI tract. Sugiuar et al. (2004) showed that in mouse colon, the functions of transient receptor potential cation channel subfamily V member 1 channels were facilitated by both 5-HT_{2A} and 5-HT_4 receptor activation. In rat colon, a synergistic link between 5-HT_4 and 5-HT_3 receptors has been suggested (Smith et al., 1999). A proposed intracellular "cross talk" between 5-HT_3 and NK_1 receptors may also provide a pathway via which certain selective 5-HT_3 receptor antagonists can influence the receptor in an allosteric manner (e.g., palonsetron) to inhibit substance P-mediated responses and thereby exert greater antiemetic activity (Rojas et al., 2014; see later).

Finally, it should be noted that 5-hydroxyindalpine, an agonist mimicking certain atypical 5-HT–mediated responses [said to be mediated by a putative 5-HT_{1P} receptor, as yet undefined as a molecular entity despite considerable research; see Galligan (2007)], facilitated peristalsis in mouse colon but had no meaningful affinity for human 5-$HT_{1A, 1B, 1D, 2A, 2B, 2C, 3, 4, 6,}$ and $_7$ receptors or for other monoamine (adrenoceptor, dopamine, and histamine) receptors (Mitchell et al., 2009). It has been speculated that at least some "atypical 5-HT responses" may be the result of ligands acting at allosteric binding sites and/or at GPCR heterodimers, such as a 5-$HT_{1B/1D}$ and dopamine D_2 receptor heterodimer (Galligan, 2007).

B. Functions of 5-HT

Of the many proposed roles for endogenous 5-HT in GI physiology, the most widely studied relate to its involvement in GI movements, secretion, reflex functions, and sensations. Several of these functions are mediated via 5-HT_3 and 5-HT_4 receptors; other receptors may also have important roles, although the available data are often limited. It is important to appreciate that GI functions can differ markedly between species, especially between rodents and humans (Sanger et al., 2011b, 2013b), and this can greatly change the functions of 5-HT and thereby complicate the translational value of certain animal models.

Additional developmental roles for endogenous 5-HT have also been reported, acting as an enteric neurotrophic or neuroprotective agent via 5-HT_{2B} and 5-HT_4 receptors or when released from enteric neurons, to promote epithelial growth via 5-HT_{2A} receptors on submucosal cholinergic neurons (Fiorica-Howells et al., 2000; Liu et al., 2009; Li et al., 2011b; Gross et al., 2012; Gershon, 2013; Mawe and Hoffman, 2013; Takaki et al., 2014). The absence of neuronal 5-HT during development in mice lacking TPH2, for example, is associated with a profound ENS hypoplasia and slow GI transit (Li et al., 2011b). Neurons that are born (become postmitotic) after enteric serotonergic neurons, in the sequence of ENS neurogenesis, are 5-HT–dependent and are particularly deficient in these animals. Thus, 5-HT is a growth factor that is required for enteric neuronal development. The mucosa was also defective in TPH2KO mice, suggesting that neuronal 5-HT promotes division of transit-amplifying cells in intestinal crypts (Gross et al., 2012). Furthermore, the normal postnatal accretion of enteric neurons and growth of mice during the first 4 months of life also does not occur in animals in which 5-HT_4 receptors have been deleted (Liu et al., 2009).

One possibility is that neuroprotective functions of neuronal 5-HT might be integrated with a proinflammatory role of mucosal 5-HT. For example, mucosal 5-HT enhances and triggers inflammation (Bischoff et al., 2009; Ghia et al., 2009; Haub et al., 2010; Li et al., 2011a; Margolis et al., 2014), probably by stimulating dendritic cells (Li et al., 2011a). Inflammation is potentially toxic to enteric neurons (Gulbransen et al., 2012), but it is also important in protecting the bowel from microbial invasion. Mucosal 5-HT may therefore enhance the strength of the innate immune response to danger, while at the same time, neuronal 5-HT may protect enteric neurons from being damaged by the response; 5-HT can thus serve both as a sword and a shield of the gut (Gershon, 2012).

1. Movements of the Hungry Stomach. During hunger, the release of 5-HT from the upper GI tract, together with the hormone motilin, has a potential role in initiating a repeating pattern of upper GI movements known as the migrating motor complex, also associated with changes in blood flow, gall-bladder emptying, and gastric and pancreatic secretions.

The migrating motor complex, which in humans repeats every 80–120 minutes, is characterized by a relatively long period of quiescence (phase I), irregular nonpropulsive movements (phase II), and then a short burst (5–8 minutes) of high-amplitude propulsive contractions (phase III) initiated by the vagus nerve in stomach, duodenum, and jejunum but rapidly recovering (phase IV) while migrating to the terminal ileum, where the movement is terminated. Phase III removes undigested material and prevents bacterial overgrowth; it can be disrupted by disease and may help develop feelings of hunger (Sanger and Lee, 2008; Sanger et al., 2011a; Deloose et al., 2012; Tack et al., 2014). In dogs (Nakajima et al., 2010), a gradual release of 5-HT from duodenal enterochromaffin cells during phase I eventually activates 5-HT_4 receptors within the myenteric plexus to increase GI motility (phase II), releasing more 5-HT from enterochromaffin cells to initiate phase III via 5-HT_3 receptors in the stomach (also Morita et al., 2013) and in humans (Wilmer et al., 1993; Luiking et al., 2002) and 5-HT_4 receptors in the intestine (dogs: Davidson et al., 1990; Nakajima et al., 2010). The vagus also increases motilin release from human mucosal

enteroendocrine cells (Wilmer et al., 1993) to re-enforce gastric phase III activity by greatly facilitating cholinergic motor nerve activity in a short-lasting manner (Broad et al., 2012).

2. Movements and Sensations of the Stomach and Duodenum after Meals. In healthy volunteers, little or no influence of endogenous 5-HT has been detected on gastric accommodation, compliance, sensation and motility, or on the rate of gastric emptying after ingestion of a meal, at least by acting at 5-HT_3 (e.g., Gore et al., 1990; Kuo et al., 2002; Netzer et al., 2002; Janssen et al., 2011b; Kusakabe et al., 2014) or 5-HT_4 receptors (e.g., Bharucha et al., 2000). In contrast, 5-HT_3 receptor antagonism can increase gastric emptying in rodents (e.g., Costall et al., 1987; Miyata et al., 1995), although an ability of ondansetron, a racemate, to facilitate cholinergic activity in guinea pig ileum via an unknown, non–5-HT_3–mediated mechanism should also be noted (Miyata et al., 1995; González and Puig, 1997). However, in the mouse, elimination of neuronal 5-HT as a result of the knockout of TPH2 accelerates gastric emptying (Li et al., 2011b), and 5-HT deletion or antagonism impairs vagal relaxation of the guinea pig stomach (Bülbring and Gershon, 1967).

If a meal is rich in glucose, amino acids, and/or lipids, high concentrations of these nutrients and/or gastric acid within the lumen of the duodenum can release 5-HT from enterochromaffin cells (and other mediators such as cholecystokinin, potentially acting synergistically together; Hayes and Covasa, 2005) to activate 5-HT_3 receptors upon abdominal vagal nerve terminals to induce satiety (Feinle and Read, 1996; Savastano and Covasa, 2007) and, if necessary, nausea (see below). In rodents, a clear reduction in gastric emptying has also been observed, but in humans, intraduodenal infusion of glucose or a high-fat meal caused only a small 5-HT_3 receptor–mediated reduction in gastric antrum movements and gastric emptying (Stacher et al., 1990; Raybould et al., 2003; Savoye et al., 2007). In rats, 5-HT_4 receptors on intrinsic cholinergic neurons may be activated to increase duodenal bicarbonate secretion for a neutralizing action against gastric acid (Akiba et al., 2015).

3. Movements of the Small Intestine. It has long been known that 5-HT is released into the intestinal lumen by mechanical stimuli to the mucosa, leading to facilitation of the peristaltic reflex (e.g., Bülbring and Lin, 1958; Foxx-Orenstein et al., 1996; (Bertrand et al., 2000, 2008; Pan and Gershon, 2000; Patel et al., 2007). However, it seems unlikely that this mechanism has a major physiologic influence on human small-intestinal movements, at least via 5-HT_3 (Gore et al., 1990; Houghton et al., 2000) and 5-HT_4 receptors (Bharucha et al., 2000). Nevertheless, this does not rule out the possibility that under certain nonphysiologic conditions (e.g., prevention of 5-HT reuptake by use of an SSRI), small-intestinal movements can be stimulated by the released 5-HT (Grover and Camilleri, 2013; Bundeff and Woodis, 2014; see *XX. C. 5-HT in GI Pathology* for discussion). Furthermore, it should be noted that 5-HT is probably released from EC cells into the lamina propria underlying these cells. Nerve fibers from intrinsic primary afferent neurons are found in this location, as are sensory nerves derived from the vagus and dorsal root ganglia. Because the entire enteroendocrine system, including EC cells, secretes basolaterally, not apically, the luminal appearance of 5-HT is likely to result from spillover (5-HT is a relatively small molecule so it can diffuse into the lumen after its release into the lamina propria). It might thus be expected that endogenous 5-HT release in the small intestine would not affect motility.

4. Movements of the Colon. Endogenous 5-HT acting at 5-HT_3 receptors plays a physiologic role in controlling normal movements of the colon via extrinsic and/or intrinsic nerve pathways, depending on the species of mammal. Additionally, and in certain species, 5-HT_{2B} and 5-HT_7 receptors may play similar roles (see below). However, the exact role of 5-HT on movements of the colon remain uncertain due, in part, to the high complexity of both the 5-HT system and the movements of colon in different species. This uncertainty has been discussed in a series of "cross talk" articles (Heredia et al., 2013; Smith and Gershon, 2015a,b; Spencer et al., 2015a,b).

a. 5-HT_{2A} receptors. 5-HT_{2A} receptor knockout mice, from which 5-HT can no longer cause contraction of the isolated colon, display apparently normal GI transit and colorectal motility patterns (Fiorica-Howells et al., 2002).

b. 5-HT_{2B} receptors. Propulsive movements of the colon and fecal output in vivo are reduced by 5-HT_{2B} receptor antagonism in rodents (Bassil et al., 2009) but not in dogs (Morita et al., 2013). The receptor has been implicated in enteric nerve and in ICC development (Fiorica-Howells et al., 2000; Tharayil et al., 2010). Although exogenously applied 5-HT can cause contraction of human colon via 5-HT_{2B} receptor activation (Borman et al., 2002), a role for endogenous 5-HT acting at the 5-HT_{2B} receptor to affect human colonic functions has yet to be established.

c. 5-HT_3 receptors. 5-HT_3 receptor antagonists slow colonic motility and induce mild constipation in several species, but the mechanisms of action may vary. In humans, the ability to slow colonic motility (e.g., Gore et al., 1990; Talley et al., 1990; Houghton et al., 2000) is at least partly due to inhibition of the gastrocolic reflex, thought to be a vagus nerve–mediated colonic contractile response associated with eating but specifically evoked by distension of the gastric antrum or inclusion of lipids within the lumen of the duodenum (Prior and Read, 1993; von der Ohe et al., 1994; Björnsson et al., 1998, 2002). In these studies, the ascending and descending components of the peristaltic reflex were unaffected by 5-HT_3 receptor antagonism. The latter is

consistent with an inability to detect 5-HT–mediated fast synaptic potentials in the myenteric plexus of human colon (Brookes et al., 1987) but seems at variance with the ability of local application of 5-HT to excite submucosal neurons (but not chloride ion secretion) in human small and large intestine via 5-HT_3 receptors (where 5-HT3A and 5-HT3B receptor subunits are expressed; Michel et al., 2005).

In rodents, 5-HT_3 receptors appear to influence colonic movements via more "local" mechanisms. Thus, the ability of 5-HT_3 receptor antagonists (granisetron and tropisetron but not ondansetron) to dose-dependently inhibit fecal pellet excretion by guinea pigs could be at least partly mimicked in guinea pig mid-to-distal isolated colon, in which granisetron and tropisetron prevented movement and expulsion of endogenous fecal pellets (recovered by application of naloxone; Sanger and Wardle, 1994). Similar data were reported by Jin et al. (1999) using artificial fecal pellets and approximately the same region of colon after application of different 5-HT_3 and 5-HT_4 receptor antagonists. However, in guinea pig distal colon, movements of artificially inserted fecal pellets were unaffected or only transiently reduced when 5-HT_4 or 5-HT_3 receptor antagonists (including granisetron and ondansetron) were applied individually but were inhibited, albeit for only a short period of time, when the antagonists were given together (Kadowaki et al., 1996). In rats, proximal colon transit (measured via an indwelling cannula) and 5-HT–induced diarrhea in mice were unaffected by 5-HT_4 or 5-HT_3 receptor antagonists (ondansetron) applied separately but were inhibited when applied together (Nagakura et al., 1997). In contrast, Yu et al. (2015) demonstrated an ability of 5-HT_3 receptor antagonism to abolish propulsive movements of rat isolated colon, again supporting a local role for 5-HT in the control of rodent colonic movements.

More recent studies confirmed a local release of 5-HT from mouse colon in response to a maintained presence of a fecal pellet, an ability of 5-HT_3 receptor activation to promote pacemaker activity generated in mouse ileum by the interstitial cells of Cajal (Liu et al., 2011), and an ability of 5-HT_3 receptor antagonism to prevent colonic migrating motor contractions (e.g., Bush et al., 2001; Heredia et al., 2009, 2013; Dickson et al., 2010). The question of whether the 5-HT comes from interneurons or from enterochromaffin cells to evoke these movements—or even if endogenous 5-HT is required at all—is the subject of debate, raising interesting questions about methods, the role of mucosal versus stretch-induced reflexes, putative constitutive expression of receptors (Smith et al., 2010, 2014; Heredia et al., 2013; Sia et al., 2013), and mouse strain differences (Neal et al., 2009). Despite the controversy surrounding the function of colonic 5-HT, myenteric 5-HT neurons project so extensively in the colon that these cells have been called "the central processing unit in the colon" (Okamoto et al., 2014).

d. 5-HT_4 receptors. 5-HT_4 receptor antagonists have little (Morita et al., 2013) or no ability to inhibit colon movements in dogs (Nagakura et al., 1996) or rodents (Kadowaki et al., 1996; Nagakura et al., 1997; Sanger et al., 1998, 2000), although reduced GI activity was observed in 5-HT_4 receptor knockout mice, arguably related to loss of 5-HT_4 receptor–mediated promotion of survival of enteric neurons (Gershon and Liu, 2007). In human volunteers, there were no changes in colonic transit (a trend toward delayed transit was not statistically significant), fasting or postprandial motor activity, compliance, or sensations evoked by transverse and sigmoid colon distension after 10–12 days administration with pharmacologically effective doses of a 5-HT_4 receptor antagonist (Bharucha et al., 2000).

e. 5-HT_7 receptors. Activation of 5-HT_7 receptors expressed by human intestinal muscle causes muscle relaxation (Prins et al., 1999; Coupar et al., 2007; Irving et al., 2007). In guinea pig ileum, 5-HT_7 receptors are localized both to muscle and to myenteric and submucosal neurons (Tonini et al., 2005). In this tissue, the release of 5-HT from enterochromaffin cells is thought to activate 5-HT_7 receptors on intrinsic sensory neurons (defined as Dogiel type II neurons) to evoke slow depolarization (Monro et al., 2005) and perhaps also to facilitate a descending inhibitory motor pathway to relax the muscle, increasing its ability to accommodate and thereby reducing the likelihood of inducing peristalsis by intraluminal distension (Tuladhar et al., 2003; Tonini et al., 2005). By a similar process, endogenous 5-HT may activate 5-HT_7 receptors in mouse colon to promote descending inhibitory interneurons and contribute to the generation of spontaneous colonic migrating motor complex (Dickson et al., 2010).

C. 5-HT in Gastrointestinal Pathology

The large amount of 5-HT in the GI tract and the expression of all 5-HT receptors on several, functionally different types of GI cells (see above) creates the interesting situation of being able to use selective 5-HT receptor antagonists to treat disease caused by release of endogenous 5-HT and also use 5-HT receptor agonists ("exogenous 5-HT") to treat other diseases. This section discusses the involvement of endogenous 5-HT in the mechanisms of disease.

Increased release or availability of 5-HT from EC cells is associated with various GI disorders, including diarrhea (e.g., induced by cholera and other bacterial toxins and also by carcinoid tumors), inflammatory bowel disease, and functional disorders such as IBS [Gershon and Tack, 2007; Spiller, 2008b, 2011; Bertrand and Bertrand, 2010; for discussion on expression of 5-HT receptors by human dendritic and immune cells, see Idzko et al. (2004) and Shajib and Khan (2015)]. More recently, the release of 5-HT from enterochromaffin cells of the rat duodenum has been shown to increase following exposure to short-chain fatty acids (Akiba et al., 2015),

potentially caused by increased TPH1 transcription (Reigstad et al., 2015). 5-HT release is also increased by cytotoxic anticancer treatments following generation of free radicals in enterochromaffin cells (Minami et al., 2003) and acute stress via agents such as corticotropin-releasing factor (Sanger et al., 2000; Von Mentzer et al., 2007). Increases in 5-HT availability in 5-HT transporter knockout mice can change the level of expression and sensitivity of enteric 5-HT_3 receptors (Gershon, 2003).

In GI disease, an increased availability of 5-HT has marked effects on certain autonomic functions (e.g., emesis), GI movements (e.g., diarrhea), and, perhaps, conscious perceptions of discomfort and/or pain. Several studies have investigated the role of 5-HT release into the circulation, 5-HT in tissue (typically by immunohistochemistry), and mRNA expression of *5-HTTLPR* gene (which determines SERT levels) in rectal mucosa and platelets and DNA polymorphisms in *5-HTTLPR* gene. In summary, the most consistent findings are elevated postprandial plasma 5-HT in IBS-D and reduced levels in IBS with constipation (IBS-C) (Spiller, 2008a,b; El-Salhy et al., 2012; Mawe and Hoffman, 2013; Camilleri, 2014; Zhang et al., 2014).

1. Emesis. There are no "universal" antiemetic drugs, and multiple stimuli evoke emesis via different pathways, not all of which involve the release of 5-HT. The stimuli that involve the release of 5-HT and that have been studied most often are discussed below. In this discussion, the term "emesis" is taken to represent the combined act of vomiting (and dry retching) as well as nausea. It is, however, increasingly appreciated that the sensation of nausea is not fully explained by the pathways that induce vomiting. For example, 5-HT_3 and NK_1 receptor antagonists are both more effective against vomiting than they are against nausea, implying that different mechanisms are involved (Andrews and Sanger, 2014) and, hence, different approaches to treatment are required (Sanger et al., 2013).

Since the pioneering studies with animals (Costall et al., 1986; Miner and Sanger, 1986; Miner et al., 1987), the use of selective 5-HT_3 receptor antagonists as antiemetic drugs has revolutionized treatment of cancer by making chemo- and radiotherapeutic treatments more tolerable (emesis is now viewed as something that can be treated rather than needs to be tolerated, and anticancer drugs can now be given in family-orientated outpatient clinics) by enabling the delivery of more aggressive treatments and by actually reducing health care costs (Currow et al., 1997; Warr and DeAngelis, 2009). Following identification of this role for the 5-HT_3 receptor, it rapidly became standard practice to coadminister a 5-HT_3 receptor antagonist with the corticosteroid dexamethasone to achieve even better relief from emesis evoked by moderate to severe emetogenic treatments. Later, the "triple-therapy" of 5-HT_3 receptor antagonism, dexamethasone, and NK_1 receptor antagonism achieved a further benefit in patients receiving treatments classified as "highly emetogenic," not only controlling the appearance of "acute" emesis (during the first 24 hours after initiation of treatment) but also, even more importantly, now controlling the "delayed emesis," which in these patients can occur 24–48 hours after the start of treatment (Warr, 2012).

5-HT_3 receptor antagonists prevent cytotoxic-associated vomiting by blocking the ability of released 5-HT (Barnes et al., 1990; Cubeddu et al., 1990), likely from EC cells, to activate 5-HT_3 receptors on abdominal vagal nerve terminals and thereby effectively "desensitize" the vagus to the proemetic stimulatory actions of other substances (e.g., prostanoids) released during the cytotoxic treatment (see Sanger and Andrews, 2006). Most recently, evidence is emerging that a long-lasting 5-HT_3 receptor antagonist (palonosetron) may provide further improvements in emesis control by a mechanism not yet clearly understood but argued to involve inhibition of substance P–mediated responses via a unique interaction with the 5-HT_3 receptor (Rojas et al., 2014).

Depending partly on the population studied, a minority of patients treated with moderate to highly emetogenic chemotherapy do not respond well to treatment with a 5-HT_3 receptor antagonist. This may be due to mutations in a P-glycoprotein efflux transporter in intestinal epithelia and capillaries of the blood-brain barrier, affecting the availability and target engagement of these and other drugs (Perwitasari et al., 2011; Tsuji et al., 2013; He et al., 2014; Zoto et al., 2015), and/or differences in rates of metabolism of ondansetron and tropisetron associated with polymorphisms of the gene encoding CYP2D6 (Kaiser et al., 2002). Genetic variants of the 5-HT_{3B}, but not the 5-HT_{3A} subunit, are also reported to alter antiemetic efficacy in a small number of patients (Tremblay et al., 2003; Kaiser et al., 2004; de Wit et al., 2005).

In patients at the end of life, perhaps with far-advanced cancer for which chemo- or radiotherapy is no longer provided, emesis can remain a severe problem for reasons associated with the use of drugs; cranial, electrolytic, or metabolic causes; and bowel obstruction, uremia, and/or sepsis. In these patients, 5-HT_3 receptor antagonists have often provided effective control of emesis (e.g., Currow et al., 1997; Mystakidou et al., 1998; Buchanan and Muirhead, 2007). Exactly where the 5-HT comes from is not always clear, and 5-HT_3 receptors expressed both peripherally (vagus nerve terminals) and centrally within the brainstem are likely to be involved (Sanger and Andrews, 2006).

SSRIs can induce nausea and vomiting that is reduced by 5-HT_3 receptor antagonism and associated with polymorphisms of the *HTR3B* gene but not with genes encoding the 5-HT transporter, the 5-HT_{2A} receptor, or the 5-HT_{3A} receptor subunit (Sugai et al., 2006; Tanaka et al., 2008). Such drugs also stimulate small but not large bowel motility and have

been evaluated as potential treatments of IBS, improving general well-being (Grover and Camilleri, 2013; Bundeff and Woodis, 2014).

Notably, there are many drugs and experimental tools other than the SSRIs that increase (e.g., 5-hydroxytryptophan, nonselective SSRIs, and monoamine oxidase inhibitors) or decrease 5-HT availability (e.g., fenfluramine; depletion following the initial increase), sometimes with additional abilities to antagonize certain 5-HT receptors, and these can induce or reduce emesis. The receptors include 5-HT_{1A}, 5-HT_{2A}, and 5-HT_3, but which receptors are involved is not always clear, and studies with more selective ligands are required to understand mechanisms of action (Johnston et al., 2014).

Postoperative vomiting (POV) is caused by multiple stimuli but can be reduced by 5-HT_3 receptor antagonism (e.g., Chun et al., 2014). The exact mechanism is not clear (Horn et al., 2014). Genetic variations in the HTR3A and HTR3B genes may be associated with the risk of developing POV, but given the multifactorial causes of POV, larger studies are required to determine the true clinical significance of these observations (Rueffert et al., 2009; Ma et al., 2013).

2. Eating Disorders. The 5-HT_3 receptor antagonist ondansetron reduces binge-eating, vomiting, and depressive symptoms in patients with severe bulimia nervosa, leading to a return of normal eating, possibly by modulating cyclic increases in vagal nerve activity (Faris et al., 2006). Arguably, an ability of 5-HT_3 receptor antagonism to increase the threshold before satiation is reached (Janssen et al., 2011b) could also play a role. Variants of the *HTR3B* gene have been associated with the restrictive subtype of anorexia nervosa (Hammer et al., 2009). It may also be possible to achieve long-term regulation of body weight by modulating different aspects of gastric motility (Janssen et al., 2011), perhaps with drugs that act at different 5-HT receptors (see below).

3. Carcinoid Diarrhea. Carcinoid tumors in the colon can generate high levels of circulating 5-HT, causing diarrhea by stimulating colonic motor functions (von der Ohe et al., 1993) via 5-HT_3 receptors (see above) and chloride ion secretion in human colon via 5-HT_{2A} and 5-HT_4 (ascending colon) and 5-HT_{2A} (descending colon) receptors (Borman and Burleigh, 1996). This is primarily treated with loperamide or a somatostatin analog, such as octreotide, but 5-HT_3 receptor antagonists have helped, at least when given acutely, as have 5-HT_{2A} (ketanserin) receptor antagonists and methysergide, a nonselective 5-HT_1/5-HT_2 receptor agonist/antagonist (Camilleri and von der Ohe, 1994; Schwörer et al., 1995; Spiller, 2008a). Given that TPH1 is responsible for 5-HT production, perhaps its inhibition may represent a new treatment paradigm in carcinoid tumors (see Camilleri, 2011).

4. Functional Gastrointestinal Disorders. These are a grouping of GI disorders that cannot be explained by structural or tissue abnormalities and as such are defined by symptoms. They include functional dyspepsia, IBS, and several others (Longstreth et al., 2006).

5. Functional Dyspepsia. The rationale for treatment of functional dyspepsia with a 5-HT receptor antagonist has not been established. Single doses of a 5-HT_4 receptor antagonist did not affect symptoms (Van Lelyveld et al., 2006), although an ability of 5-HT_4 receptor antagonism to prevent HCO_3^- release in rat duodenum by SCFAs has suggested a role when high levels of SCFAs occur in the upper GI tract during bacterial overgrowth (Akiba et al., 2015). More promisingly, a pilot study with patients dosed for 12 weeks with a 5-HT_3 receptor antagonist showed some improvement in "adequate relief of pain or discomfort" (Talley et al., 2001), and an association between symptoms and 5-HT3A receptor gene polymorphism has been suggested (Mujakovic et al., 2011). Arguably, these data find some consistency with an ability of 5-HT_3 receptor antagonism to prevent the experience of nausea in healthy volunteers induced by intraduodenal infusion of lipids [see Gershon and Tack (2007)], as early satiety and nausea are common symptoms in this group of patients (Vanheel et al., 2013) but are at variance with the lack of ability of 5-HT_3 receptor antagonism to change sensitivity to gastric distension [see Gershon and Tack (2007)].

6. Irritable Bowel Syndrome. In some pilot studies, 5-HT_3 receptor antagonism reduced sensations caused by bowel distension in patients with IBS [e.g., Prior and Read (1993) and Goldberg et al. (1996)], perhaps as a consequence of increased compliance to distension (Delvaux et al., 1998). These studies, together with the known ability to reduce colonic movements, prompted the evaluation and initial success of the 5-HT_3 receptor antagonist alosetron as a treatment for patients with IBS with symptoms of diarrhea as well as abdominal discomfort and/or pain. The subsequent occurrence of ischemic colitis in a small number of treated patients restricted use of alosetron and almost entirely stopped research into this area. It has since been concluded that ischemic colitis is two- to four-times more likely to occur in patients with IBS regardless of treatment (Lewis, 2011), suggesting that any future treatments of this form of IBS should not induce severe constipation to potentially exacerbate such a liability. Furthermore, it should be noted that an ability of 5-HT_3 receptor antagonists to reduce sensations evoked by bowel distension has not been consistently observed [e.g., Hammer et al. (1993) and Zighelboim et al. (1995) in patients without "psychologic disorders"), perhaps arguing for greater emphasis of future research on colonic movement disorders. Any association between IBS and *HTR3* subunit gene mutations remains uncertain

(Niesler, 2011). Nevertheless, in patients with diarrhea-predominant IBS, the ability of alosetron to reduce colonic transit may be associated with long (LL) polymorphisms of the 5-HT transporter gene *5-HTTLPR,* which is associated with increased synthesis of SERT (SLC6A4) and inactivation of endogenous 5-HT (Camilleri et al., 2002). Also, in this group of patients with IBS, symptoms were improved in a pilot study using the 5-HT_3 receptor antagonist ramosetron, an activity correlating with increased expression of TPH1 and with *TPH1* gene polymorphism (Shiotani et al., 2015).

The 5-HT_4 receptor antagonist SB-207266 reduced stress-induced defecation in mice (Sanger et al., 2000), and in a pilot study with diarrhea-predominant IBS patients, it tended to reduce rectal sensitivity and reduced small-intestinal transit (Houghton et al., 1999). However, lack of significant efficacy in larger studies stopped further development for this indication (De Ponti, 2004).

In some countries, the 5-HT_4 receptor agonist tegaserod was registered for treatment of IBS and chronic constipation (see below for discussion on the potential use of 5-HT_4 receptor agonists in the treatment of IBS) but was then withdrawn because of potential cardiovascular liability and poor overall efficacy (Schiller and Johnson, 2008). Tegaserod has since been shown to act as a potent 5-HT_{2B} receptor antagonist (Beattie et al., 2004), reducing colonic motility (in rodents, not dogs; see above) and exerting a visceral antinociceptive activity in rodents (Ohashi-Doi et al., 2010; O'Mahony et al., 2010). The extent to which each of these different activities translates to humans and/or patients with IBS is not clear. In parallel with the evidence that LL polymorphisms of the 5-HT transporter gene *5-HTTLPR* result in greater changes in colonic transit with alosetron, a clinical trial has shown that LL polymorphism is associated with reduced clinical efficacy of tegaserod in patients with constipation (Li et al., 2007).

LX1031, a locally acting TPH inhibitor, that does not cross the blood-brain barrier, has been found to be safe and well tolerated in an exploratory 4-week phase 2 study in patients with symptomatic, nonconstipating IBS (Brown et al., 2011). Thus, reduction of mucosa-derived 5-HT may positively influence symptoms common to nonconstipating IBS. A relationship was observed between symptom improvement and a reduction in 24-hour urinary 5-HIAA; thus, 5-HIAA can serve as a biomarker to estimate the rate of 5-HT synthesis and target engagement by the TPH inhibitor LX1031. However, data from any further clinical development of this drug is lacking, and the company Lexicon have confirmed development has been terminated.

Any involvement of the 5-HT_7 receptor in the mechanisms of visceral pain, following a proposed role in somatic pain (Andrews and O'Neill, 2011), is as yet unknown. Similarly, any involvement of 5-HT in the mechanisms of mucosal inflammation (see earlier) in patients with IBS has yet to be demonstrated.

7. Other Gastrointestinal Disorders. In colon from patients with diverticular disease, 5-HT_4 mRNA expression was reduced in the muscle but increased in the mucosa; expression of 5-HT_{2B} and 5-HT_3A mRNA was unchanged. The authors speculate that these changes could influence the intestinal motor disturbances associated with this disease (Böttner et al., 2013).

D. Therapeutic Benefits of 5-HT Receptor Agonists and Antagonists

This section discusses the use of 5-HT receptor agonists ("exogenous 5-HT") to treat diseases where any involvement of endogenous 5-HT in the mechanism of the disease is absent or unclear. The class of 5-HT_3 receptor antagonists (e.g., alosetron or ondansetron) for IBS-D and 5-HT_4 receptor agonists for chronic constipation or IBS-C (e.g., prucalopride) are extensively used in clinical practice and constitute first- or second-line therapeutic agents for these conditions. Efficacy and safety are documented by systematic reviews and meta-analyses (Andresen et al., 2008; Ford et al., 2009; Shin et al., 2014). The risk of ischemic colitis with alosetron (not observed with ondansetron) is estimated at about 1 in 1000 patients; however, there is also evidence that IBS itself is a risk factor for the development of ischemic colitis (Huerta et al., 2011).

1. 5-HT_4 Receptor Agonists. At present, 5-HT_4 receptors appear to have little or no major roles in disorders of GI hypomotility; for example, in idiopathic gastroparesis, there were no overall changes in 5-HT_4 receptor expression apart from a reduced expression of the 5-$HT_{4(c)}$ splice variant (van Lelyveld et al., 2008), yet 5-HT_4 receptor agonists have long been used to treat such disorders. This began with metoclopramide, a derivative of procaine found to have surprising antiemetic and gastric prokinetic properties. Understanding the mechanisms of action of metoclopramide directly led to the discovery of the antiemetic role of the 5-HT_3 receptor (Miner and Sanger, 1986) and in the description of a novel "5-HT–like" receptor affecting GI motility (Sanger, 1987b), later named as the 5-HT_4 receptor by Bockaert and colleagues who used similar ligands in CNS studies (Dumuis et al., 1988). Subsequently, a number of different 5-HT_4 receptor agonists were launched as prokinetic agents, but none of the early examples are selective in their action, leading to cardiovascular complications (Sanger, 2009). Nevertheless, the selective 5-HT_4 receptor agonist prucalopride is now marketed as a treatment of chronic idiopathic constipation; others are in development, and the potential use of such agents in the treatment of upper GI disorders associated with gastric and/or esophageal hypomotility is still being explored (Sanger, 2009; Broad et al., 2014a,b; Kessing et al., 2014). Interestingly, a low dose of the 5-HT_4 receptor agonist

mosapride has been shown to increase human gastric accommodation in healthy volunteers (Amano et al., 2015), perhaps reflecting the ability of 5-HT_4 receptor agonists to increase nitrergic as well as cholinergic activity (Cellek et al., 2006), the former potentially increasing gastric fundus accommodation and the latter, gastric antrum motility and emptying. Because impaired gastric accommodation may contribute to the etiology of symptoms in patients with postprandial functional dyspepsia (e.g., early satiety and/or nausea; Talley, 2015), these data support the argument that such symptoms can be relieved by selective 5-HT_4 receptor agonists (Janssen et al., 2011a; Sanger et al., 2013).

In human colon, 5-HT_4 receptor activation is an effective prokinetic principle, as it facilitates excitatory cholinergic and inhibitory nitrergic motor nerve activities (representing the ascending excitatory and descending inhibitory components of a peristaltic reflex), decreases muscle tension, and increases chloride secretion from the mucosa into the lumen (Borman and Burleigh, 1996; Prins et al., 2000; Cellek et al., 2006; Broad et al., 2013). Evidence for facilitation of enteric sensory nerve activity remains uncertain (Gershon and Tack, 2007). More recent attention has been drawn to the possibility that 5-HT_4 receptor activation might stimulate bicarbonate secretion in rat proximal colon (Kaji et al., 2015) and might also reduce nociception in rats exposed to colorectal distension (Hoffman et al., 2012; Gilet et al., 2014). The latter activity could depend on a synergistic interaction with 5-HT_3 receptors in the mechanisms of allodynia (Smith et al., 1999) but also contrasts with the findings of Sugiuar et al. (2004), who showed that the nociceptive functions of transient receptor potential cation channel subfamily V member 1 channels were facilitated by 5-HT_4 receptor activation in mouse colon. Similar responses have not yet been confirmed to occur in human colon, but if successfully translated, 5-HT_4 agonists may be useful as suppositories or enemas for treating IBS (Kale-Pradhan and Wilhelm, 2007; Mawe and Hoffman, 2013). To date, only the 5-HT_4 receptor agonist tegaserod has been fully tested as a potential treatment of IBS, but poor efficacy at the 5-HT_4 receptor, potential confounding cardiovascular complications, and the existence of additional ability to antagonist at the 5-HT_{2B} receptor (see above for discussion) greatly complicate the use of such data to argue for or against the use of 5-HT_4 receptor agonists as a treatment of any form of IBS.

Finally, the possibility that 5-HT_4 receptor activation might promote neurogenesis in adults has received support from studies using mice (increased bromodeoxyuridine incorporation into neurons, neural precursors, or stem cells; Liu et al., 2011), guinea pigs, and rats (regeneration of neural circuitry or recovery of reflex activity after rectal transection and anastomosis, accompanied by increased neurofilament and neural stem cell markers; Takaki et al., 2014), suggesting potential use of 5-HT_4 receptor agonists in treatments of disorders associated with intestinal aganglia.

2. Other 5-HT Receptor Agonists. Different 5-HT_{1A} receptor agonists, including buspirone, may reduce emesis induced by different stimuli in animals, including cisplatin and motion, although species differences in actions complicates the interpretation of data (Johnston et al., 2014). In patients with functional dyspepsia, repeat-dosing with the 5-HT_{1A} receptor agonist R137696 did not reduce dyspeptic symptoms or gastric accommodation (Tack et al., 2009).

When given acutely after meals to patients with functional dyspepsia, the 5-$HT_{1B/1D}$ receptor agonist sumatriptan delayed gastric emptying, improved gastric accommodation, and reduced the perception of gastric distension and early satiety (Tack et al., 2004). The suggestion that this receptor may be involved in the mechanisms of vomiting has not yet been resolved (Johnston et al., 2014), especially as sumatriptan may act on the yet to be defined 5-HT_{1P} receptor.

Partial 5-HT_3 receptor agonists (e.g., pumosetrag; CSTI-300) have been identified for treatment of patients with diarrhea-predominant IBS (e.g., Moore et al., 2013; Roberts et al., 2020). CSTI-300 displays comparable efficacy to alosetron in a rat model of colon distension (Roberts et al., 2020). Pumosetrag has been evaluated in patients with gastroesophageal reflux disease (Choung et al., 2014), reducing the rate of acid reflux events but not symptoms.

A summary of some 5-HT drugs in development for GI therapeutics is summarized in Table 22 and reviewed elsewhere (Valentin et al., 2015).

XXI. 5-HT Receptors and the Immune System

A. Introduction

The defense against pathogens is mediated by innate and adaptive immune mechanisms that act in the periphery and the CNS. 5-HT regulates inflammation and immunity by acting on 5-HT receptors that are differentially expressed on immune cells, both in rodents and humans. 5-HT acts as a potent chemoattractant, recruiting innate immune cells to sites of inflammation. 5-HT also alters the production and release of cytokines and cell activation/proliferation. Some immune cells, including mast cells and T lymphocytes, have the capacity to synthesize and release 5-HT, expanding the range of tissues for 5-HT signaling.

B. How Do Immune Cells Encounter 5-HT?

Although 5-HT is largely studied as a neurotransmitter, enterochromaffin cells of the gut produce most of the body's 5-HT that functions as a local hormone. These cells express tryptophan hydroxylase TPH1, a rate-limiting enzyme for 5-HT production (Walther et al., 2003). A second TPH isoform, TPH2, synthesizes 5-HT

TABLE 22
Some novel 5-HT agents for GI indications

Drug Class	Example	Putative Action in Humans	Pharmacodynamic in Humans	Clinical Efficacy: Phase IIB or III	Safety Issues, Approval, Other
TPH_1 blocker	LX-1031	Inhibits synthesis of 5-HT by blocking TPH_1	Inhibition of urine 5-HIAA excretion; no studies of PD efficacy	Phase IIB trial in non–C-IBS: 1000-mg dose, improved adequate relief, stool consistency	Reported to be discontinued
5-HT_3 receptor antagonist	Ramosetron	Inhibits secretion, motility, nociception	ND	Phase IIB 5- and 10-μg dose studies in IBS-D: benefit on global relief and bowel function	Approved in Asia, under investigation elsewhere; ischemic colitis with same drug class
5-HT_4 receptor agonist	Prucalopride	Selective 5-HT_4 receptor agonist; stimulates colonic motility	Accelerated CT in health and CC	Multiple phase II/III trials completed; open label experience of ~1000 cumulative patient-years; efficacy in CC (males and females in phase III and IV clinical trials), with consistent benefits shown by meta-analyses	No clinical cardiac AEs in clinical trials of >4000 humans; approved in virtually all countries except United States
5-HT_4 receptor agonist	Velusetrag	Selective 5-HT_4 receptor agonist; stimulates colonic motility	Accelerated CT in health in dose-related fashion	Phase IIB efficacy; no effect on QT in health or 400 patients with constipation	Under investigation (although last report 2017)
5-HT_4 receptor agonist	Naronapride	Selective 5-HT_4 receptor agonist; stimulates colonic motility	Accelerated CT in health	Under investigation	Under investigation (although last report 2018)
5-HT_4 receptor agonist	Relenopride	Selective 5-HT_4 receptor agonist; stimulates colonic motility	Accelerated CT in functional constipation	Phase II studies ongoing in IBS-C patients (ClinicalTrials.gov trial NCT02082457).	May be discontinued (last active report 2015)

AEs, adverse events; CC, chronic constipation; CT, colonic transit; ND, not determined.

in the CNS and gut enteric nerves (Walther et al., 2003). 5-HT concentrations in blood and tissues are normally kept relatively low. Immune cells, however, may encounter 5-HT released in the gut mucosa or from platelets that sequestered 5-HT via the 5-HT transporter SERT (SLC6A4). In turn, platelets can release accumulated 5-HT at sites of injury and inflammation. Platelet-derived 5-HT is important for attracting innate immune cells such as neutrophils to inflamed tissue (Duerschmied et al., 2013). In addition to platelets, dendritic cells (professional antigen-presenting cells) and B lymphocytes express SERT and, thus, accumulate and release 5-HT. Interestingly, recent studies indicate that some immune cells are capable of 5-HT biosynthesis. Mast cells (tissue-resident cells) in rodents and humans express TPH1, and levels of 5-HT are elevated in patients with mastocytosis, who have greatly elevated mast cell numbers (Kushnir-Sukhov et al. 2007; Nowak et al., 2012). Furthermore, T lymphocytes (O'Connell et al., 2006; León-Ponte et al. 2007; Urbina et al., 2014) also express TPH1 upon mitogen or T-cell receptor activation and can synthesize 5-HT. Interestingly, expression of TPH1 and 5-HT production is greater in $CD8^+$ compared with $CD4^+$ T cells (Chen et al., 2015).

C. 5-HT and Hematopoiesis

It has been proposed that 5-HT acts at hematopoietic stem cell progenitors directly or via modulation of the bone marrow microenvironment (Yang et al., 2007). Mice deficient in peripheral 5-HT ($Tph1^{-/-}$) display morphologic and cellular features reminiscent of ineffective erythropoiesis (Amireault et al., 2011). Other data show that the bone marrow composition of $Htr_{2B}^{-/-}$ mice displays a significant increase in Cd11b+/Gr+ cells that represents granulocyte precursors. This is associated with a significant reduction in Cd11b-/Cd31+ population that corresponds to immature endothelial progenitor cells in $5\text{-}HT_{2B}^{-/-}$ mice (Launay et al., 2012). These observations support the hypothesis that 5-HT signaling controls the differentiation of myeloid precursor cells, particularly in the monocyte/macrophage lineages.

D. 5-HT and the Immune Tolerance

One well documented way to control immunity and tolerance is through the regulation of nutrients in the microenvironment of immune cells. Best described is tryptophan deficiency mediated by the catabolic enzyme indoleamine 2,3-dioxygenase (IDO), which locally depletes tryptophan and liberates immunoregulatory metabolites known as kynurenines. T-cell activation is exquisitely sensitive to local tryptophan catabolism, and thus this enzyme exerts profound protective effects in allo-fetal rejection, autoimmunity, and inflammation (Munn and Mellor, 2013). Although IDO is thought to be the major tryptophan-catabolizing enzyme outside of the liver, TPH1 shares a similar K_M to IDO (~20 μM) (Mckinney et al., 2005) and can also

potentially exhaust tryptophan to regulate immune tolerance. Indeed, in models of skin allograft tolerance, tumor growth, and experimental autoimmune encephalomyelitis (multiple sclerosis), *Tph1* deficiency was shown to break allograft tolerance, to induce tumor remission, and to intensify neuroinflammation independent of the downstream product 5-HT (Nowak et al., 2012).

E. 5-HT and the Innate Immune Response

Innate immune system function involves monocytes, macrophages, dendritic cells, neutrophils, eosinophils, mast cells, and natural killer cells that act immediately in the area of infection, leading to the destruction of pathogens. Innate immunity is primarily responsible for recognizing and eradicating "nonself" molecules presented by pathogens and is therefore confined to recognizing extracellular pathogens (bacteria vs. viruses). This response is nonspecific with respect to particular invaders but provides immediate host defense against pathogens via pattern recognition by toll-like receptors (TLRs). Pathogen-associated molecular patterns (e.g., peptidoglycans, bacterial LPS, and double-stranded viral RNAs) bind TLRs on antigen-presenting cells, namely, dendritic cells and macrophages. Antigen-presenting cells then phagocytose pathogens and display pathogen-derived peptides via the major histocompatibility complex on their cell surface for recognition by leukocytes of the "adaptive" immune system. Antigen-presenting cells also secrete proinflammatory cytokines (e.g., IL1β, IL-6, and TNFα), prostaglandins, and histamine, which further activate physiologic responses, alerting the body to infection/invasion. In addition to cellular protective mechanisms, innate immunity also includes the complement system, activated by foreign substances, antigen-antibody complexes (classic pathway), and Gram-negative bacteria (alternative pathway). This system leads to cell lysis, increased vascular permeability (allowing antibodies, innate immune cells, and fluid to enter tissues), and chemotaxis. The complement system also helps to activate antigen-presenting cells, namely, dendritic cells and B cells, during specific immune responses. Innate immunity also functions to communicate the presence of pathogens to cells involved in adaptive immune responses (Baganz and Blakely, 2013). The local environment and the presence of stimulatory signals determine whether monocytes acquire dendritic cell or macrophage characteristics and functions. 5-HT receptors are expressed by a broad range of inflammatory cell types, including monocytes, macrophages, and dendritic cells.

Neutrophils are the most abundant white blood cell and serve an essential role in innate immunity, particularly against bacteria. Duerschmied et al. (2013) reported that $Tph1^{-/-}$ mice show mild leukocytosis (e.g., elevated white blood cells) numbers compared with WT mice, primarily driven by an elevated neutrophil count. Despite this, 50% fewer leukocytes rolled on unstimulated mesenteric venous endothelium of $Tph1^{-/-}$ mice. Diminished rolling in $Tph1^{-/-}$ mice resulted in reduced firm adhesion of leukocytes after LPS treatment, and neutrophil extravasation into lung, peritoneum, and skin wounds was reduced in $Tph1^{-/-}$ mice. 5-HT alone did not induce neutrophil migration in vitro, suggesting that endothelial adhesion was the primary deficit. Consequently, survival from LPS-induced endotoxic shock was improved in $Tph1^{-/-}$ mice. In conclusion, platelet 5-HT promotes the recruitment of neutrophils in acute inflammation (Duerschmied et al., 2013); however, the nature of the 5-HT receptors underlying these effects is unknown.

In human CD14 monocytes, mRNA expression of 5-HT_{1E}, 5-HT_{2A}, 5-HT_3, 5-HT_4, and 5-HT_7 receptors has been revealed (Dürk et al., 2005). 5-HT modulates the release of IL-1β, IL-6, IL-8/CXCL8, IL-12p40, and TNF-α, whereas it has no effect on the production of IL-18 and IFN-γ in LPS-stimulated human blood monocytes. Moreover, 5-HT modulates mRNA levels of IL-6 and IL-8/CXCL8 but not of IL-1β and TNF-α. Pharmacologic experiments suggested that signaling through the 5-HT_3 receptor upregulates the LPS-induced production of IL-1β, IL-6, and IL-8/CXCL8 but not that of TNF-α and IL-12p40. Furthermore, activation of the Gs-coupled 5-HT_4 and 5-HT_7 receptors increases secretion of IL-1β, IL-6, IL-12p40, and IL-8/CXCL8, but in contrast, it inhibits LPS-induced TNF-α release. Interestingly, 5-HT_{1E} and 5-HT_{2A} receptor agonists do not modulate the LPS-induced cytokine production in human monocytes (Dürk et al., 2005). Instead, 5-HT modulates cytokine production via activation of 5-HT_3, 5-HT_4, and 5-HT_7 receptors.

5-HT has been shown to upregulate the activity of peritoneal macrophages and to increase the in vitro activity of phagocytosis in a concentration-dependent manner via 5-$HT_{1A/7}$ receptors and NF-κB (Freire-Garabal et al., 2003). Gene expression profiling of proinflammatory M1 (granulate-macrophage colony-stimulating factor) and anti-inflammatory M2 (macrophage colony-stimulating factor) macrophages revealed that 5-HT_{2B} and 5-HT_7 receptor mRNAs are preferentially expressed by M2 macrophages, whereas the 5-HT_7 receptor is the only 5-HT receptor expressed in M1 macrophages (de Las Casas-Engel et al., 2013). The 5-HT_{2B} receptor is preferentially expressed by anti-inflammatory M2 macrophages and is also detected in vivo in liver Kupffer cells and in tumor-associated macrophages. Expression of 5-HT_{2C} receptors was also reported in alveolar macrophages, where 5-HT induces a rise in intracellular Ca^{2+} concentration and an increased expression of CCL2 (monocyte chemoattractant protein-1) mRNA (Mikulski et al., 2010). LPS, the archetypal macrophage-activating stimulus that

signals via TLR4, was shown to regulate the expression of 5-HT_{2B} receptors in mouse macrophages. 5-HT_{2B} receptor mRNA is increased 20-fold in murine thioglycollate-elicited peritoneal macrophages (Lattin et al., 2008). 5-HT was shown to inhibit the LPS-induced release of proinflammatory cytokines, to upregulate expression of macrophage M2 polarization–associated genes, and to reduce the expression of M1-associated genes. Only 5-HT_7 receptors mediate the inhibitory action of 5-HT on the release of proinflammatory cytokines. Both 5-HT_{2B} and 5-HT_7 receptors mediate the pro-M2 skewing effect of 5-HT. Blockade of both receptors during in vitro monocyte-to-macrophage differentiation preferentially modulates the acquisition of M2 polarization markers (de Las Casas-Engel et al., 2013).

In mice, it has been established that 5-HT is an important regulator of microglia, the brain resident macrophages, which derive from yolk sac hematopoietic stem cell precursors. In the presence of 5-HT, the microglial processes moved more rapidly toward a lesion, which is considered a chemotactic response. Similarly, the chemotactic response of cultured microglia to ATP is enhanced by 5-HT. Phagocytic activity determined by the uptake of microspheres reveals that 5-HT application decreases phagocytic activity of amoeboid microglia. The presence of microglial 5-HT_{2B}, 5-HT_{5A}, and 5-HT_7 receptors was confirmed by patch-clamp experiments in culture and amoeboid microglia and by qPCR analysis of RNA isolated from primary cultured and acutely isolated adult microglia (Krabbe et al., 2012). This was recently confirmed by two-photon microscopy, showing that microglial processes moved rapidly toward the source of 5-HT via activation of the 5-HT_{2B} receptor (Kolodziejczak et al., 2015). Modulation of microglial functions such as phagocytosis and migration is fundamental for the CNS, as microglia can influence the balance of synaptogenesis and neuronal death during development and in pathology.

Platelet activation was reported in patients with various allergic disorders. Platelet-derived factors may influence monocytic differentiation into dendritic cells. Indeed, 5-HT alters differentiation of monocytes into dendritic cells (triggered by granulocyte-macrophage colony-stimulating factor and IL-4), leading to dendritic cells with reduced expression of costimulatory molecules and CD1a and higher expression of CD14. These 5-HT–triggered dendritic cells exhibit significantly reduced stimulatory activity toward allogeneic T cells. However, they show enhanced cytokine-producing capacity, including for IL-10 but not IL-12. 5-HT–induced alteration of the dendritic cells phenotype and the reduction in antigen-presenting capacity are mediated via 5-HT_{1E}/5-HT_7 receptors (Katoh et al., 2006).

Immature dendritic cells preferentially express mRNA for 5-HT_{1B}, 5-HT1E, and 5-HT_{2B} receptors, whereas mature dendritic cells mostly express 5-HT_4 and 5-HT_7 receptors. The mRNA expression level of the ligand-gated cation channel 5-HT_3 and the GPCR 5-HT_{2A} receptors are not modified during maturation. 5-HT stimulates 5-HT_3–dependent Ca^{2+} influx in both immature and mature dendritic cells. The 5-$HT_{1B/1E}$ and 5-$HT_{2A/2B}$ receptor stimulation induces intracellular Ca^{2+} mobilization via Gi/Gq proteins in immature, but not mature, dendritic cells. Activation of 5-$HT_{4/7}$ receptors induces cAMP elevation in mature dendritic cells. Functional studies indicate that activation of 5-HT_4 and 5-HT_7 receptors enhances the release of the cytokines IL-1β and IL-8 while reducing the secretion of IL-12 and TNF-α in mature dendritic cells (Idzko et al., 2004).

5-HT is able to induce oriented migration in immature but not in LPS-matured dendritic cells via activation of 5-$HT_{1B/1E}$ and 5-$HT_{2A/2B}$ receptors. Accordingly, 5-HT also increases migration of pulmonary dendritic cells to draining lymph nodes in vivo. By binding to 5-HT_3, 5-HT_4, and 5-HT_7 receptors, 5-HT upregulates the production of the proinflammatory cytokine IL-6. Additionally, 5-HT influenced chemokine release by human monocyte–derived dendritic cells: production of the potent T-helper cells Th1 chemoattractant IP-10/CXCL10 was inhibited in mature dendritic cells, whereas CCL22/ macrophage-derived chemokine secretion was upregulated in both immature and mature dendritic cells. Furthermore, dendritic cells matured in the presence of 5-HT switched to a high IL-10– and low IL-12p70–secreting phenotype. Consistently, 5-HT favored the outcome of a Th2 immune response both in vitro and in vivo (Müller et al., 2009). A recent study using $Htr7^{-/-}$ mice confirmed 5-HT_7 receptor expression in $CD103^+CD11c^+$ dendritic cells found in colon (and spleen) and its importance in immune activation and gut inflammation (Kim et al., 2013).

Interestingly, like platelets, dendritic cells can take up 5-HT from the microenvironment, and the antidepressant fluoxetine inhibits this uptake. Expression of 5-HT transporters (SERTs) is regulated by dendritic cell maturation, exposure to microbial stimuli, and physical interactions with T cells. Significantly, 5-HT sequestered by dendritic cells is stored within LAMP-1+ vesicles and subsequently released via Ca^{2+}-dependent exocytosis, as confirmed by amperometric recordings (O'Connell et al., 2006).

5-HT is chemotactic for eosinophils. Notably, allergic asthma is characterized by infiltration of eosinophils, and plasma levels of 5-HT are elevated in symptomatic asthma patients. There is solid evidence that 5-HT contributes to this eosinophil recruitment. Indeed, 5-HT alone can stimulate in vitro migration of murine and human eosinophils (Boehme et al., 2004; Kang et al., 2013). Although several 5-HT receptor subtypes are expressed, 5-HT_{2A} is the most prominent, and 5-HT_{2A} receptor antagonists inhibit 5-HT–induced but not eotaxin-induced migration. Furthermore, eosinophils roll in response to 5-HT in venules under conditions of

physiologic shear stress (Boehme et al., 2004; Kang et al., 2013). Signaling via 5-HT_{2A} receptors is associated with changes in cell shape/morphology via activation of specific intracellular signaling molecules (ROCK, MAPK, PI3K, and the PKC-calmodulin pathway) (Kang et al., 2013).

Mast cells have the capacity to synthesize and accumulate 5-HT (Kushnir-Sukhov et al., 2007). In turn, this stored 5-HT can be released upon IgE cross-linking. Furthermore, mast cells express mRNA for multiple 5-HT receptors, including 5-HT_{1A}, 5-HT_{1B}, 5-HT_{1D}, 5-HT_{2A}, 5-HT_{2B}, 5-HT_6, and 5-HT_7 receptors (Kushnir-Sukhov et al., 2006). 5-HT can induce mast cell adherence to fibronectin and stimulate cell migration. However, there is no evidence that 5-HT degranulates mast cells or modulates their activation by IgE. Mast cells from the 5-HT_{1A} receptor knockout mouse ($Htr_{1A}^{-/-}$) do not respond to 5-HT, indicating a principal role for this receptor. Importantly, 5-HT attracts mast cells to sites of inflammation; injection of 5-HT into the skin enhances the accumulation of mast cells in wild-type but not in 5-HT_{1A} receptor–null mice.

Natural killer cells are large lymphocytes with innate killing capacity. Addition of 5-HT to mixtures of target cells and $CD56^+$ natural killer–enriched human mononuclear cells strongly augmented natural killer cell cytotoxicity via 5-HT_{1A} receptors. This effect was indirect and involved 5-HT signaling at accessory monocytes. The cytotoxicity-enhancing effect of 5-HT was additive to that induced by IFN-α, IFN-γ, or IL-2 but not to histamine (Hellstrand and Hermodsson, 1987).

F. 5-HT and Adaptive Immunity

The response of a second immune system division, termed the adaptive, or specific, immune system, occurs within hours of an infection and involves antigen-specific recognition and destruction of pathogens by T and B lymphocytes. The two components of the adaptive immune system involve cell-mediated and humoral immunity. Cell-mediated immunity is carried out by T cells located in the thymus, lymph nodes, and circulation. Antigen-presenting cells that migrate to lymph nodes will prime and educate T cells as to the nature of the pathogen. T cells then proliferate and differentiate into, for example, $CD4^+$ T-helper inflammatory cells (Th1) that activate macrophages, $CD4^+$ Th2 cells that aid antibody responses, or $CD8^+$ cytotoxic cells that target cells infected with intracellular microbes. The second component of adaptive immunity involves the contributions of B cells, located in lymph tissue, spleen, and in the circulation. Upon stimulation, B cells become plasma cells (with or without the help of Th2) that produce and secrete antibodies (immunoglobulins). Memory T and B cells recognize specific antigens and respond quickly. Thus, the adaptive immune system is distinguished from the innate immune system by its ability to identify, remember, and eliminate pathogens that have been designated as nonself. Adaptive immunity is triggered at the immune synapse, where peptide major histocompatibility complexes and costimulatory molecules expressed by dendritic cells are physically presented to T cells (Baganz and Blakely, 2013).

The mRNA expression of 5-HT receptors in lymphoid tissues of the rat, ex vivo isolated spleen, thymus, and peripheral blood lymphocytes include 5-HT_{1B}, 5-HT_{1F}, 5-HT_{2A}, 5-HT_{2B}, 5-HT_6, and 5-HT_7 receptor mRNAs. Mitogen-stimulated spleen cells additionally expressed mRNA corresponding to the 5-HT_3 receptor (Stefulj et al., 2000). In the rhesus macaque, SERT-positive cells were found among $CD4^+$, $CD3^+$, and $CD3^+CD4^+$ lymphocytes, respectively (Yang et al., 2007). Fluoxetine significantly increases the number of lymphocytes expressing SERT and stimulates an enrichment of $CD8^+$ T cells, decreasing the $CD4^+/CD8^+$ ratio. Fluoxetine administration elevates the levels of IL-4 at 1, 2, and 3 weeks and of IL-2 at 2 and 3 weeks. The IL-4/IL-2 ratio is significantly increased in fluoxetine group compared with the controls and is similar during the 3 weeks of treatment (Fazzino et al., 2009).

There is long-standing evidence that 5-HT can influence T-cell activation. Notably, mice treated with a selective, irreversible inhibitor of TPH1, parachlorophenylalanine, exhibit a reduction in the number of CD25-positive T cells (Young et al., 1993; León-Ponte et al., 2007), suggesting that 5-HT contributes physiologically to T-cell activation. A screen for 5-HT receptor subtypes in murine T cells revealed expression of three subtypes; naive T cells selectively express 5-HT_7 receptors, whereas following T-cell activation, there is a strong upregulation of 5-HT_{1B} and 5-HT_{2A} receptors (León-Ponte et al. 2007). Significantly, exogenous 5-HT induces rapid phosphorylation of ERK1/2 and IκBα in naive T cells that is inhibited by preincubation with a selective 5-HT_7 receptor antagonist. Thus, 5-HT signaling via the 5-HT_7 receptor may contribute to early T-cell activation. Yin et al. (2006) showed that 5-HT_{1B} receptor antagonists impaired the proliferation of helper $CD4^+$ T cells in mouse and human. Inoue et al. (2011) showed that a 5-HT_{2A} receptor agonist enhanced Concavalin-A–induced activation of murine $CD4^+$ and $CD8^+$ T cells, whereas a 5-HT_{2A} receptor antagonist blocked T-cell receptor–mediated IL-2 and interferon-γ production. Consistent with these data, Akiyoshi et al. (2006) showed that treatment with a 5-HT_{2A} receptor antagonist enhanced the survival of cardiac allograft in mice. Thus, these mouse data strongly support involvement of 5-HT receptors (5-HT_7, 5-HT_{1B}, and 5-HT_{2A}) during early- and late-stage T-cell activation.

Interestingly, although not detected in mouse, the 5-HT_{2B} receptor is found in human T cells. Gene expression profiles during human $CD4^+$ T-cell differentiation identified the 5-HT_{2B} receptor with 10-fold greater expression in $CD3^{high}CD4^+CD8^-$

SP4 thymocytes over intrathymic T progenitor cells, $CD3^-CD4^+CD8^+$ "double positive" thymocytes, $CD3^+CD4^+CD8^-CD45RA^+CD62L^+$ "naive" T cells from cord blood, and $CD3^+CD4^+CD8^-CD45RA^+CD62L^+$ "naive" T cells from adult blood (Lee et al., 2004). Furthermore, 5-HT_{2B} receptors are differentially expressed among Th subsets. In human umbilical cord blood, Th cells cultured in the presence of cytokines promoting Th2 differentiation were found to increase 5-HT_{2B} receptor expression along with 50 Th2 differentially expressed genes (Aijö et al., 2012).

5-HT may also modulate migration of human T cells. Human but not mouse T cells express functional 5-HT_3 receptors. 5-HT_3 receptor agonists selectively decrease T-cell migration toward gradients of the chemokine CXCL12 but not to other chemokines such as CCL2 and CCL5. Interestingly, CXCL12 is highly expressed on vascular endothelium and inhibits T-cell migration across endothelium and extravasation. In transmigration experiments, 5-HT_3 receptor stimulation reverses this effect of endothelial-bound CXCL12 on T-cell migration (Magrini et al., 2011). These data suggest that 5-HT can stimulate trafficking of T cells from blood to tissues.

T cells have the capacity to synthesize 5-HT, and levels of TPH1 expression increase following T-cell activation (O'Connell et al., 2006; León-Ponte et al. 2007; Urbina et al., 2014; Chen et al., 2015). The precise signaling role for T-cell–produced 5-HT is uncertain. Conceivably, TPH1 activity in T cells could act to exhaust tryptophan, as has been proposed for mast cells (Nowak et al., 2012). On the other hand, 5-HT produced by T cells might act in an autocrine or paracrine manner. Indeed, T cells express the type 1 vesicular monoamine transporter responsible for vesicular storage of 5-HT, and type 1 vesicular monoamine transporter expression increases following T-cell activation concomitant with TPH1. Furthermore, Ca^{2+} elevations in T cells can trigger secretion of 5-HT. Interestingly, levels of TPH1 and monoamine oxidase A, the principal catabolic enzyme for 5-HT, are greater in $CD8^+$ compared with $CD4^+$ T cells, suggesting a specific biologic role for 5-HT synthesis in this T-cell subset (Chen et al., 2015). B lymphocytes have the capacity to sense and sequester 5-HT via SERT. 5-HT increases mitogen-stimulated $CD19^+$ B lymphocyte proliferation in a concentration- and time-dependent manner. These effects are reproduced by a 5-HT_{1A} receptor agonist. 5-HT–induced increases in proliferation are blocked by 5-HT_{1A} receptor antagonists. Moreover, LPS-activated mouse spleen cells express specific binding sites for 5-HT_{1A} receptor, suggesting that 5-HT upregulates mitogen-stimulated B lymphocyte proliferation through 5-HT_{1A} receptors (Iken et al., 1995). Furthermore, mitogen-activated B lymphocytes express higher levels of 5-HT_{1A} receptor mRNA and protein than resting cells. This upregulation is seemingly dependent on NF-κB transcription factors, as selective inhibitors of this pathway prevent the increase in mRNA expression for the 5-HT_{1A} receptor (Abdouh et al., 2001).

B lymphocytes express SERT, and uptake of 5-HT leads to apoptosis of Burkitt lymphoma cells (Serafeim et al., 2002). 5-HT may induce apoptosis via the intracellular serotonylation signaling pathway. Furthermore, long-term treatment with SSRIs in humans leads to enhanced (~30%) numbers of B lymphocytes (Hernandez et al., 2010). Interestingly, higher doses of SSRIs directly promote apoptosis of Burkitt lymphoma cells by inhibiting DNA synthesis, whereas normal peripheral and tonsilar B cells are relatively resistant to SSRI-induced apoptosis (Serafeim et al., 2003). SERT has been detected in a variety of B-cell lines (Meredith et al., 2005), revealing SERT as a potential target for a broad range of B-cell malignancies.

XXII. General Summary and Conclusions

The first official IUPHAR review on 5-HT receptors (Hoyer et al., 1994) was a landmark for the then rather complex 5-HT receptor field: it has come of age and has been cited well over 3600 times (Google Scholar). It followed a number of initiatives and meetings in the late 1980s, when the 5-HT receptor nomenclature committee was established (by our esteemed colleague and friend Paul Vanhoutte in 1987, who sadly died in 2019). The committee was constituted and met formally for the first time in 1990 at the occasion of the 5-HT meeting in Basel (a satellite to the 1990 IUPHAR main meeting); a number of recommendations were made adapting to the new findings in transduction mechanisms and molecular biology of the receptors over the subsequent decade (Humphrey et al., 1993a; Hoyer et al., 1994, 2002; Hartig et al., 1996; Hoyer and Martin, 1997; Martin et al., 1998). It is remarkable that the recommendations made at the time have been largely accepted by a community that was used to very different nomenclatures or even definitions of receptors and that very little needed to be changed or added to these recommendations (Alexander et al., 2015a,b,c; 2019). In the 1994 review, it was noted that the authors had a cumulated 100 years of active 5-HT research to share. A number of our colleagues have in the meantime retired from active research or have moved to other professional priorities. There is a lot of new "blood" now on board to reflect the growing diversity of the research, which is currently performed in many different academic and SME (small or medium enterprise) pharmaceutical centers; the combined years in 5-HT research accumulated by the authorship has increased because of the considerably greater number of authors on the present paper to address a more diverse range of complex issues.

What has clearly changed, though, is the relative representation from the industry, which compared with the 1994 version, is very much reduced. In 1994, there were six out of the eight authors who worked in "Big Pharma," whereas there are none for the present

review. This is explained by the lesser interest for exploratory 5-HT research in the industry on the one hand, with less emphasis on 5-HT translational research and a shift of research toward small or medium enterprise/Biotech; on the other hand, there is an increased interest in very basic aspects of 5-HT research, such as structural biology or the more recent advances in the immunologic aspects, which were hardly addressed in the previous review of 1994. The shift in emphasis and thus authorship was very much needed, as the 5-HT receptor field has become more complex and possibly less the subject of "classical" pharmacologists as it was in the latter decades of the last century.

The apparent good news is that no new receptors have been identified, except for some additional 5-HT_3 receptor subunits whose function still remain to be defined clearly. On the other hand, there have been major advances with respect to 5-$HT_{1B/1D}$ receptors; as far as can be told, all triptans act as 5-$HT_{1B/1D}$ receptor agonists, and in the meantime, many different triptans have reached the market primarily for the acute treatment of migraine. Some of them, such as sumatriptan, may also act as 5-HT_{1F} receptor agonists. Thus, the 5-HT_{1B} receptor is most probably the main and sole target for triptans in the treatment of migraine, whereas the 5-HT_{1D} receptor, which is comparatively less abundant, may only play a minor role. Indeed, a study in migraine with a selective 5-HT_{1D} receptor agonist (PNU142633) was not conclusive; it can be argued that the compound was only a partial agonist and that target engagement may not have been optimal. Thus, the jury is still out to define a role for the 5-HT_{1D} receptor in physiology and disease. Because of the triptans, of which many starting with sumatriptan have affinity, the 5-HT_{1F} receptor has attracted quite some attention, and there is clinical evidence (with lasmiditan) that this constitutes another target for the treatment of migraine. This is especially true for patients who want to avoid vascular side effects, as in contrast to the 5-HT_{1B} receptor, the 5-HT_{1F} receptor is not expressed in vascular tissues. The 5-HT_{1A} receptor is still actively investigated, especially because newer highly selective 5-HT_{1A} receptor ligands show very different patterns of pathway selectivity and thus may have clinical application in diseases as different as chronic pain, Parkinson disease, or Rett Syndrome and other forms of autistic disorders/mental retardation, as illustrated by the relatively recent FDA approval for compounds such as flibanserin, cariprazine, vortiotexine for the treatment of female sexual desire deficit, schizophrenia, and depression, respectively. Although these compounds have other activities, they all share 5-HT_{1A} receptor agonism with possible differences in receptor engagement in different brain regions. An interesting aspect with some of the newer 5-HT_{1A} receptorligands is their variety of activities at different transduction systems (i.e., these compounds have various degrees of biased agonism or signaling). This is not unique to 5-HT_{1A} receptors, as probably most 5-HT GPCR agonists show different levels of biased signaling depending on their preferential activation of one or the other multiple combinations of receptor/G protein and accessory proteins. This has become even more evident now that crystal structures exist for both 5-HT_{1B} and 5-HT_{2B} receptors, which show that various ligands can occupy different conformation in the same orthosteric pocket, which may explain why certain 5-HT_{2A} receptor agonists produce hallucinations when others do not. In addition, it has become evident that the orthosteric binding pocket is not the unique target for ligands, since a number of ergolines (e.g., ergotamine or DHE) may bind an accessory (allosteric site?), which has been well described for some metabotropic GPCRs (e.g., mGlu or $GABA_B$ receptors), opening the possibility for allosteric 5-HT receptor modulators (some of which have already been proposed for, for example, 5-$HT_{1B/1D}$ or 5-HT_3 receptors).

The aspect of species differences is a recurrent theme, as illustrated by the amply documented and well accepted differences in the pharmacology of 5-HT_{1B} receptors (explained by a single amino acid change in the core structure of the ligand-binding site) or the multiple differences observed with splice variants of the 5-HT_4 or 5-HT_7 receptors. A potential function for the 5-ht_{1e} receptor in native tissue or cells is still to be identified; absence from rodents hinders the research and has made 5-ht_{1e} receptor drug discovery relatively unattractive in the absence of a link to disease. A similar point can be made about the 5-HT_5 receptors, in which both a relative lack of tools and the absence of a link to disease also hinder progress, although recent advances in knowledge concerning the 5-HT_{5A} receptor may promote further research. However, as the 5-ht_{5B} receptor appears to be nonfunctional in humans, this likely prevents interest as a therapeutic target.

Significant progress has been made in the 5-HT_2 receptor family. The potential role played by 5-HT_{2B} receptors in valvulopathies led to many of the drugs acting as agonists at these receptors being withdrawn from the market since the early 2000s (fenfluramine, norfenfluramine, benfluorex, and pergolide), keeping in mind that MDMA ("ecstasy") and its active metabolites act as 5-HT_{2B} receptor agonists, as may also be the case for a number of "designer" drugs. As a consequence, there is significant emphasis on screening for 5-HT_{2B} receptor agonism in preclinical safety studies and the "red flag" on further clinical development of such compounds. It has been established that the 5-HT_{2C} receptor comes in a variety of edited forms (up to 24 possible variants in the human brain) that may be linked to disease (e.g., depression, suicide, impulsivity, addiction, spinal cord injury, and obesity as induced by some antipsychotic drugs). The edited forms of the receptor can display different coupling ability, lower affinity for agonists, and lower constitutive activity

compared with the nonedited isoform that can couple to multiple G proteins. In addition, both agonists (lorcaserin) and antagonists (agomelatine) have been/are being developed to treat obesity/schizophrenia/addiction and depression/generalized anxiety disorder, respectively. Interestingly, in spinal cord injury, the 5-HT_{2C} receptor is less edited and expression is increased, resulting in constitutive activity and muscle spasms in the absence of any endogenous 5-HT, which can be reversed by inverse 5-HT_{2C} receptor antagonists. The 5-HT_{2A} receptor may be less investigated, as early claims that linked 5-HT_{2A} to hypertension or sleep did not result in clinical success. However, it should be kept in mind that all three, 5-HT_{2A}, 5-HT_{2B}, and 5-HT_{1B}, receptors play a role in pulmonary hypertension, a disease with unmet need for treatment, as neither endothelin antagonists nor PDE inhibitors are highly effective, although commonly prescribed. On another note, 5-HT_{2B} receptors appear to play a significant role in fibrosis, be it in the lung, liver, and especially the heart. 5-HT_{2A} receptor antagonism is a major component of second-generation antipsychotics (in combination with dopamine D_2 receptor antagonism and often many other pharmacological activities, e.g., blonanserin), and there is evidence that a 5-HT_{2A} receptor antagonist may show efficacy in certain subpopulations of schizophrenic patients. It may also be relevant that the 5-HT–glutamate link in schizophrenia may be related to the existence of heterodimers between 5-HT_{2A} and $mGlu_2$ receptors, although in vivo proof of concept remains to be established. Along these lines, there is evidence that 5-HT_{2A} receptors are able to form homodimers, similarly to 5-HT_{1B} or 5-HT_{1D} receptors; however, the latter two may also form heterodimers, which may explain the codistribution that has been consistently observed for these two receptors. The 5-HT_{2C} receptor is also capable of dimerization in vitro; it may even form tetra or octamers, and it may form heteromers with the ghrelin or the melatonin 2 receptor.

Clearly, receptor homo- and heterodimerization is an ongoing subject of interest with respect to 5-HT receptors. In addition to information incorporated within relevant sections of this review, the reader is also directed to specialized papers on this subject (e.g., Lee et al., 2000; Herrick-Davis, 2013; Moreno et al., 2016; Moutkine et al., 2017; Maroteaux et al., 2019).

In the 5-HT_3 receptor field, similarly to what has been long known and accepted for other members of the Cys-loop ligand-gated ion channel superfamily (e.g., nicotine acetylcholine receptors), homomeric and heteromeric 5-HT_3 receptors are evident, and new receptor molecular isoforms are still being investigated. Clinically, alosetron has been developed for the treatment of IBS with diarrhea, in contrast to 5-HT_4 receptor agonists, for which the primary indication remains IBS with constipation or primary constipation in spite of the withdrawal of tegaserod from most markets. Obviously, the antiemetic effects of 5-HT_3 receptor antagonists, whether in the short term or in the longer term following chemotherapy or possibly surgically induced, are still a very salient feature of these drugs, even if, in the meantime, combination therapy (with, e.g., NK1 receptor antagonists) becomes the accepted treatment. There has been a lot of research dealing with 5-HT_6 receptor antagonists in memory/dementia (e.g., idalopirdine), although the phase III clinical data has been disappointing, whereas the 5-HT_7 receptor is somewhat less actively investigated as a clinical target. The mystery of the putative 5-HT_{1P} receptor in the gastrointestinal tract remains, although heterodimerization remains a favored potential explanation, albeit without clear direct evidence.

The more troubling news is that whereas receptors used to be defined based on structural, operational, and transductional features, it becomes clear that some refinements are needed:

1) In terms of structural knowledge, progress has been relatively slow. X-ray diffraction data from crystals exist for the 5-HT_3 receptor and also, for example, the 5-HT_{1B} and 5-HT_{2B} receptors. One of the limitations of this approach for GPCRs is that the conformation of the ligand-receptor complex is very much dependent on the G protein heterotrimer associated with the receptor. In more recent years, we have learned that many receptors are able to couple to various pathways and even are able to signal in the absence of G proteins. In addition, there are multiple GPCR-interacting proteins that may affect both signal transduction and receptor-ligand conformation, thus the pharmacological signature.
2) The operational definition of a receptor (pharmacological profile based on rank orders of affinity of agonists and antagonists), which was the primary feature of receptors up to the 1990s, is now recognized as increasingly complex because of the dependence on the G protein associated with the receptor under study and other GPCR-interacting proteins. Indeed, a number of drugs will have different affinities and potencies depending on the transduction system studied. Such variations may seem artificial and linked to the expression of receptors and transduction components in engineered cells, but we know that an endogenously expressed receptor can react very differently to a given ligand in a cell- and system-dependent manner (e.g., 5-HT_4 receptors in the GIT). Thus, the concept of functional selectivity or biased agonism/antagonism is a reality (see so-called β-blockers, which have rather different clinical features depending on their signaling characteristics, e.g., carvedilol compared with propranolol, pindolol, and nadolol). In contrast to other receptors, such as

muscarinic or GABA receptors, there is little research addressing allosteric modulation for 5-HT receptors, with the possible exception of the 5-HT_3 receptor. However, it becomes clear from X-ray structural studies performed with some ergolines that in addition to the classic orthosteric binding pocket, some 5-HT receptors have an extended binding "site," which is very reminiscent of that described for muscarinic allosteric ligands. Such molecular targets may offer attractive strategies for novel therapeutics.

The relatively recently recognized importance of the microbiome continues to grow (Gilbert et al., 2018), and one aspect of this relevant to the present paper is the regulation of 5-HT receptors by bacteria-derived metabolites (e.g., Yano et al., 2015; Bhattarai et al., 2017; Cohen et al., 2017); understanding the "physiologic" and "pathologic" consequences of such modulation will no doubt provide impetus for developing therapeutic strategies harnessing these mechanisms.

In conclusion, it is a clear understatement to say a lot has been learned since 1994, but still some old questions remain unanswered, and many new questions will keep 5-HT researchers busy for years to come. Although 5-HT / serotonin / enteramine was only discovered and characterized a little more than 70-80 years ago (Viali and Erspamer, 1933; Rapport et al., 1947, 1948; Erspamer and Asero, 1952; Twarog and Page, 1953), 5-HT, its receptors, enzymes, transporters and multiple accessory proteins, constitute one of the oldest transmitter systems, estimated to have originated about 800 million years ago; hence it had time to develop a certain level of complexity. The appreciated complexity of the 5-HT system relates less to the number of 5-HT receptor classes, families, and subtypes, which was, per se, a conceptual challenge for some researchers in the 1980s and 1990s, than to the actual complex nature of the ligand/receptor/G protein/arrestin and/or other interacting protein complexes that vary according to species, organ, cells, gender, and disease state. This intricacy renders the pharmacological definition of a given receptor, or a ligand, challenging, as it may all depend on the nature and environment of the supramolecular complex, which is being specifically addressed.

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