Review

Serotonin Receptors

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Chem. Rev., **2008**, 108 (5), 1614-1641 • DOI: 10.1021/cr078224o • Publication Date (Web): 14 May 2008

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David E. Nichols*,† and Charles D. Nichols‡

Department of Medicinal Chemistry and Molecular Pharmacology, School of Pharmacy and Pharmaceutical Sciences, Purdue University, West Lafayette, Indiana 47906-2091, and Department of Pharmacology and Experimental Therapeutics, Louisiana State University Health Sciences Center, 1901 Perdido Street, New Orleans, Louisiana 70112

Received October 19, 2007

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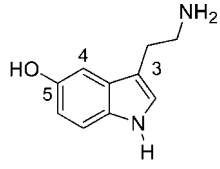
1. Introduction

Serotonin, 5-hydroxytryptamine (5-HT),



Serotonin, 5-HT

is one of the class of monoamine neurontransmitters, all of which have a chemical template comprised of a basic amino group separated from an aromatic nucleus by a two carbon aliphatic chain. In mammals, 5-HT is biosynthetically derived by two enzymatic steps: (1) ring hydroxylation of the essential amino acid tryptophan by tryptophan hydroxylase, the rate-limiting step,[1] and (2) side chain decarboxylation by aromatic amino acid decarboxylase (Figure 1). A second isoform of tryptophan hydroxylase was identified in 2003 by Walther et al.[2,3] The original enzyme originally characterized, which is expressed in the gut, is now called *tph1*, and the isoform that is expressed exclusively within the brain is named *tph2*.[4,5] In the brain, serotonin is produced within axon terminals, where it is released in response to an action potential and then diffuses across the synapse to activate postsynaptic receptors. The serotonin receptor family is larger than any other family of G-protein coupled (GPCR) neurotransmitter receptors: 13 distinct genes encoding for receptors of the G-protein coupled seven-transmembrane class. In addition, there is one ligand-gated ion channel, the 5-HT_3 receptor.

Serotonin is one of the most ancient signaling molecules. It is found in the single-celled eukaryotes paramecium and tetrahymena, where it can modulate swimming behavior and growth.[6,7] Serotonin receptors that share significant orthology are found in a very diverse range of organisms up the evolutionary tree, from planaria, *Caenorhabditis elegans*, and *Drosophila melanogaster* to humans. From this diversity, it has been speculated that the primordial serotonin receptor of the rhodopsin−GPCR family may have first appeared more than 700−750 million years ago, a time that likely predates the evolution of muscarinic, dopaminergic, and adrenergic receptor systems.[8] GPCRs as a protein family are believed to have evolved about 1.2 billion years ago.[8] Significantly, serotonin receptors appear to be among the oldest receptors within the rhodopsin-like family.[9] The three major classes of G-protein-coupled 5-HT receptors, the 5-HT_{1A}, 5-HT_2, and 5-HT_7-like receptors, which are less than 25% homologous, likely differentiated approximately 600−700 million years ago, before the time period during which vertebrates diverged from invertebrates. The fruit fly, *Drosophila melanogaster*, expresses functional orthologs of the 5-HT_{1A}, 5-HT_2, and 5-HT_7 receptors, as well as orthologs for many other GPCRs.[10] The mammalian 5-HT receptor subtypes have further differentiated over the past 90 million years.

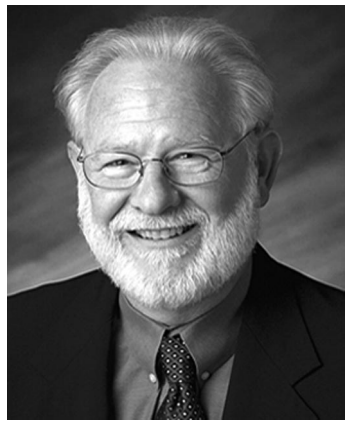
Not surprisingly, as a result of this long evolutionary history, serotonin plays a variety of roles in normal physiology, including developmental, cardiovascular, gastrointestinal, and endocrine function, sensory perception, behaviors such as aggression, appetite, sex, sleep, mood, cognition, and

* To whom correspondence should be addressed. Mailing address: Dept. of Medicinal Chemistry and Molecular Pharmacology, School of Pharmacy and Pharmaceutical Sciences, 575 Stadium Mall Drive, West Lafayette, IN 47906-2091. Tel: 765-494-1461. Fax: 765-494-1414. E-mail: drdave@pharmacy.purdue.edu.

† Purdue University.

‡ Louisiana State University Health Sciences Center.

10.1021/cr078224o CCC: $71.00
Published on Web 05/14/2008



David E. Nichols received his B.S. degree in chemistry from the University of Cincinnati, and his Ph.D. degree in Medicinal Chemistry from the University of Iowa in 1973. He then did postdoctoral work in Pharmacology with John P. Long in the College of Medicine at the University of Iowa. He began his academic career as an Assistant Professor at Purdue University in 1974, rising through the ranks to become Professor in 1984. He is Professor of Medicinal Chemistry and Molecular Pharmacology and adjunct Professor of Pharmacology at the Indiana University School of Medicine. Between 1994 and 1996, he was the Interim Chairman of both the Departments of Pharmacology and Toxicology and Medicinal Chemistry and Pharmacognosy at Purdue University and coordinated the merger of the two departments into the present Department of Medicinal Chemistry and Molecular Pharmacology. He was the cofounder and a chief scientific officer for DarPharma, Inc., a North Carolina biotech startup to commercialize dopamine D1 agonists discovered in his laboratory, until it was acquired by BioValve. In 2004, he was named the Irwin H. Page Lecturer by the International Serotonin Club, and in 2006 he received the first Provost's Outstanding Graduate Mentor award from Purdue University. In 2007, he was named the Robert C. and Charlotte P. Anderson Distinguished Chair in Pharmacology. He is a fellow of the American Pharmaceutical Association and the American Association of Pharmaceutical Scientists. His work has been continuously funded by NIH for nearly 30 years, he has published 260 refereed publications, book chapters, and monographs and has seven issued U.S. patents. His present research includes development of receptor-selective agonists for the D1 dopamine receptor, as well as development of ligands selective for the 5-HT_{2A} receptor.

Charles D. Nichols earned his B.S. degree in Biological Sciences at Purdue University in 1989. He went on to obtain his Ph.D. degree at Carnegie Mellon University in 1997, where he studied visual system development and genetics of the fruit fly *Drosophila melanogaster*. He then took a position as a postdoctoral fellow in the laboratory of Dr. Elaine Sanders-Bush, in the Pharmacology Department at Vanderbilt University, where he studied serotonin neuropharmacology. In 2002, he was appointed Research Assistant Professor at Vanderbilt, and in 2004, he was recruited to the Department of Pharmacology and Experimental Therapeutics at the Louisiana State University Health Sciences Center in New Orleans where he is currently an Assistant Professor. His research focuses on the use of both mammalian and *Drosophila*-based systems to understand serotonergic function in the central nervous system.

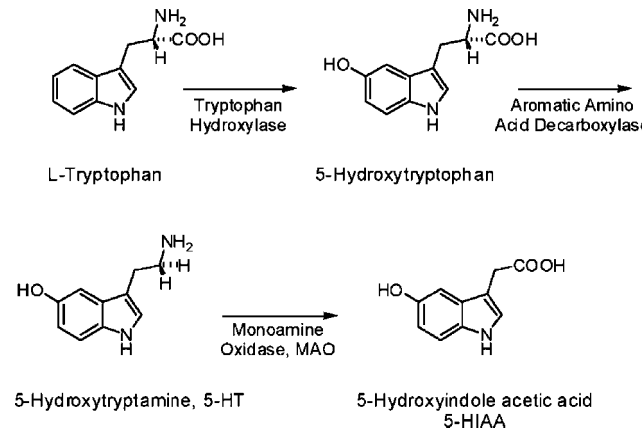


Figure 1. Biosynthesis and metabolism of serotonin. Serotonin is produced in a two-step process from the essential amino acid L-tryptophan. First, in the rate-limiting step, tryptophan hydroxylase produces 5-hydroxytryptophan (5-HTP). In the second step, aromatic amino acid decarboxylase decarboxylates the side chain to produce serotonin. A principle route of metabolic degradation for serotonin is deamination of the side chain by monoamine oxidase (MAO), principally the MAO-A isoform of the enzyme.

memory.[11] Most of the serotonin in mammals is found within the gut, produced principally by enterochromaffin cells. It is also stored within blood platelets, and this relatively large pool enabled its isolation and structure elucidation. Only later was it found in the central nervous system, where it has proven to have a number of varied and extremely important functions. In mammalian species, serotonin in the brain arises from specialized groups of cell bodies known as the raphe nuclei, located in the brainstem reticular formation. The role of serotonin in specific brain regions will be discussed when the particular receptor types are described that are located in that area.

1.1. The Discovery of Serotonin

The discovery of serotonin and its identification as an important neurotransmitter is a very interesting detective story that involved a multidisciplinary approach by several groups.[12,13] The earliest work on serotonin was carried out by Vittorio Erspamer in Rome, Italy, who had discovered that an acetone extract of enterochromaffin cells from gastrointestinal mucosa contained a substance that caused contraction of the smooth muscle of the rat uterus. On the basis of several simple chemical tests, he concluded that this substance was an indole and named it enteramine.[14] Several papers were published on enteramine by Erspamer and his group in the subsequent years, until 1952, when it was established that the active component in enteramine was identical to a substance named serotonin that had just been identified by Maurice Rapport, Arda Green, and Irvine Page.

Irvine Page was the Director of the Division of Research at the Cleveland Clinic and had been interested in the isolation of vasoconstrictor substances in the blood that might be responsible for hypertension. His laboratory had discovered that when blood coagulated, a vasoconstricting substance was immediately produced. This material was isolated and purified by Arda Green, a rather remarkable biochemist,[15] and Maurice Rapport, a talented organic chemist. Rapport later described the purification of serotonin from approximately "900 liters of serum collected from almost two tons of beef blood" over the course of his structure elucidation work.[16] The substance was ultimately purified,

crystallized, and named serotonin in 1948, the name being derived from the fact that the substance was produced in the serum ("ser"), and constricted or increased tone ("tonin") in blood vessels.[17,18] A classical chemical structure elucidation approach led Rapport to propose that serotonin was 5-hydroxytryptamine (5-HT).[18] Proof of the structure came with the chemical synthesis of serotonin in 1951 by Hamlin and Fischer at the Abbott Laboratories.[19] Erspamer and Asero also then prepared synthetic serotonin and confirmed that it was identical to enteramine isolated and purified from natural sources.[20] The synthetic material then became available for investigators and thus began an intense era of rapid investigation of the many important physiological functions for serotonin.

A role for serotonin up to that point had only been discovered in the peripheral vascular system as a substance that contracted smooth muscle and constricted blood vessels. Betty Twarog, a Ph.D. candidate at Harvard University, was working on the neurotransmitters that contracted or relaxed the byssus retractor muscle of the edible mussel. She became intrigued by the reports from Rapport's laboratory and obtained a sample of serotonin from Abbott Laboratories. She found that it contracted the muscle, and then went on to develop an extremely sensitive bioassay for serotonin using the isolated heart of the hard-shell clam *Venus mercenaria* ("quahogs"). Working in Irvine Page's laboratory at the Cleveland Clinic, she prepared acetone extracts of various mammalian tissues, including brain, and, using the clam heart assay, quantified the approximate amount of serotonin in the tissues.[21] She found readily detectable levels of serotonin in the brains of dogs, rats, and rabbits, results that were quite surprising to Irvine Page.

The finding of serotonin in the brain ultimately was catapulted to much greater significance by the discovery of the potent mind-altering properties of lysergic acid diethylamide (LSD-25) only a few years earlier. This famous discovery in 1943 by Dr. Albert Hofmann, working at the Sandoz Laboratories in Basel, has been described in detail by Hofmann himself.[22] The first systematic investigation of LSD in humans was carried out in the Psychiatric Clinic at the University of Zurich by Werner A. Stoll.[23] At about that time, Sandoz Laboratories began supplying LSD (Delysid) to psychologists and psychiatrists as a substance described to produce a model psychosis and as a potential aid in psychotherapy. Additional clinical reports using LSD began to appear in 1949, and there followed a very rapid escalation of research interest in LSD.

It was quickly realized that the tryptamine fragment embedded within the structure of LSD also was the scaffold for serotonin. It was in this context that Woolley and Shaw[24] first proposed that the "mental disturbances caused by lysergic acid diethylamide were to be attributed to an interference with the action of serotonin in the brain." This hypothesis may seem rather insignificant today, but at that time, there was no discipline of neurochemistry, and furthermore, whether mental illnesses such as schizophrenia were related to brain chemistry or even had a biological basis at all was still controversial! In a later paper, Shaw and Woolley reported that in some assays, LSD had effects resembling serotonin.[24] Whether or not the effects of LSD were related to blocking the effects of serotonin, or mimicking them, was not nearly as important as pointing out that disturbances in brain chemistry could be related to aberrant behavior and psychiatric disorders. This idea had profound effects on neuroscience and was the beginning of the era of modern neuropsychopharmacology. A rapidly increasing interest in the role of serotonin in behavior began, which has continued unabated up to the present time. Our understanding of a variety of psychiatric disorders and mood disturbances has depended in a great many cases on elucidating the role of serotonin and in studies of the functions of the various types of receptors with which serotonin interacts.

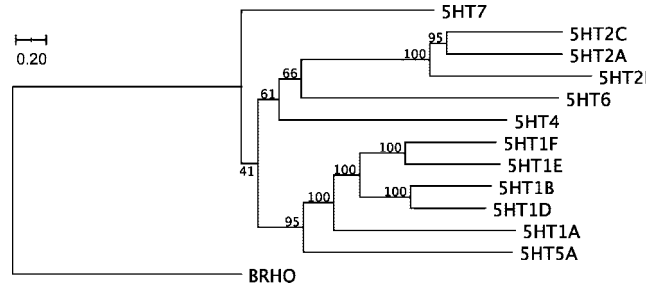


Figure 2. Scaled phylogenetic tree comparing all human serotonin receptors with bovine rhodopsin (BRHO). Results of bootstrap analysis with 100 replications are given above the branches. The scale bar corresponds to 0.2 substitutions per position for a unit branch length. The tree was constructed using the most current NIH Entrez sequence for each receptor with CLC Free Workbench software (CLC bio, Cambridge, MA).

We now know that serotonin plays a number of very important roles in normal brain function, which include modulation of mood states, hunger, sex, sleep, memory, emotion, anxiety, endocrine effects, and many others. Serotonin receptors are widely expressed throughout the brain and in many key structures responsible for cognition and basic brain functions. As one of the most ancient neurotransmitter systems, having appeared very early in evolution, its functions have been conserved and even expanded up through the various branches of the evolutionary tree.

1.1.1. Phylogeny of Serotonin Receptors

After the first 5-HT receptor was cloned (the 5-HT_{1A} receptor was the first of the many serotonin receptors to be cloned and characterized),[25] it became clear that the mammalian family of serotonin receptors was large, and indeed it has proven to be much larger than that of any of the other GPCR-type neurotransmitter receptors, including those for dopamine, norepinephrine, glutamate, or acetyl choline. Fourteen different receptor subtypes, grouped into seven families, have now been described. That classification does not include the multiple receptors generated by alternative splicing of single genes or editing of the receptor RNA.[26] The phylogenetic relationship of each receptor to the others is shown in Figure 2.

1.1.2. General Structural Features: Homologies with Rhodopsin

Except for the 5-HT_3 receptor, which is a ligand-gated ion channel, all of the other serotonin receptors are members of the G-protein coupled receptor family. The GPCR-type serotonin receptors, as well as a large number of monoamine and other neurotransmitter receptors, are classified as "type A" family, rhodopsin-like receptors.[27] Several high-resolution structures have been obtained for bovine rhodopsin, and very recently the first structure for a monoamine receptor, the β_2-adrenergic receptor, has been reported at 2.4 Å resolution.[28–30]

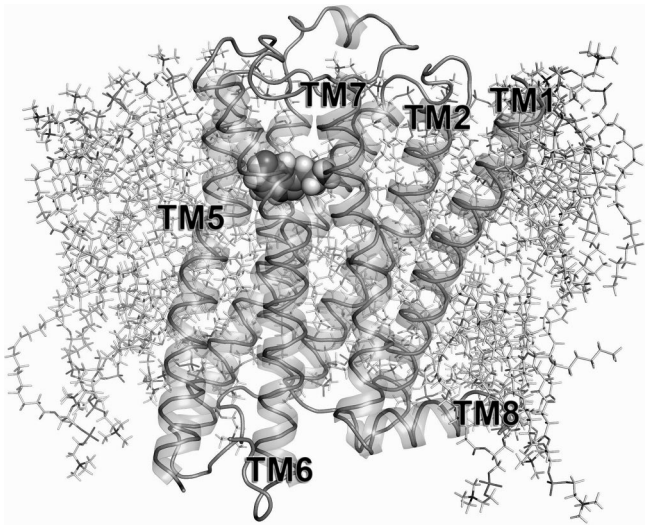


Figure 3. A model of serotonin docked into the binding domain of a homology model of the serotonin 5-HT_{2A} receptor, developed from the recently published crystal structure of the β_2-adrenergic receptor. The seven transmembrane helix motif is illustrated as transparent gray helices superimposed on the backbone ribbon with helices 5, 6, and 7 toward the front. Serotonin is shown as a space-filling model, and portions of the lipid membrane are shown as stick models around the helical bundle. The extracellular region is at the top of the figure, with the intracellular region at the bottom. G-protein coupling occurs on the intracellular side of the receptor.

This watershed event signals that perhaps the structures of other GPCRs will be forthcoming in the future.

The nature and location of the absolutely conserved residues in rhodopsin, in the β_2-adrenergic receptor, and other type A GPCRs, strongly suggests that serotonin receptors bear a high structural and functional resemblance. Although the overall homology of GPCRs compared with rhodopsin is only about 35%,[31] the presence of highly conserved ("fingerprint") motifs within the seven transmembrane regions[32] was strong evidence for an evolutionary relationship; the crystal structure of the β_2-adrenergic receptor has now strengthened that assumption. Although helical tilt, twist, and relative orientations may differ slightly within the individual GPCRs, the helical bundle is probably held together in a similar overall arrangement, and the activation mechanism likely involves similar conformational changes. Figure 3 is a representation of serotonin bound within a homology model of the serotonin 5-HT_{2A} type receptor, embedded in a model bilipid membrane. The model is based on the orientation of the helices in the crystal structure of the β_2-adrenergic receptor.[30] It should be noted that the structure of the β_2-adrenergic receptor was solved with an inverse agonist bound, rather than an agonist, so this homology model does not precisely represent the activated state of the receptor that would be observed with serotonin actually bound. Nevertheless, it is sufficient to give a good graphical representation of the relative shape and orientation of type A GPCRs and serotonin receptors in particular. This comment is made more relevant by the finding that the structure of photoactivated rhodopsin is not substantially different from the dark inverse agonist form.[33]

Important conserved features to recognize that provide a basis for this assumption will now be discussed, using the Ballesteros and Weinstein numbering system to designate the most conserved residue in each helix as X.50, where X is the transmembrane helix number.[34] The discussion will briefly highlight important residues in each helix, starting with helix 1 and proceeding through helix 8. The intent of this discussion is to present the reader with sufficiently detailed structural information about serotonin receptors (and GPCRs in general) to enable a general conceptual understanding of receptor function and signal generation. Based on homologies among all of the type A GPCRs, this discussion also can be extended to a general understanding of the other monoamine GPCRs. All of the receptor illustrations were generated with PyMol (DeLano Scientific, San Carlos, CA; http://www.pymol.org).

This discussion should be read in conjunction with inspection of Figure 4, showing the sequence alignments between the human GPCR serotonin receptors and bovine rhodopsin, the reference molecule. Conserved residues, as well as other important regions are identified in that figure. Although many of the motifs described here have been discussed by others, there are several others that, to the best of the authors' knowledge, have not been commented upon and have not been studied in mutagenesis experiments. Some of these became evident only after the structure of the β_2-adrenergic receptor was solved. The reference molecule is the most recent crystal structure of rhodopsin, at 2.2 Å resolution,[35] with additional insights gained from detailed examination of the recent crystal structure of the β_2-adrenergic receptor at 2.4 Å resolution.[30]

Important residues in transmembrane helices 1 and 2 (Figure 5) include the absolutely conserved asparagine Asn1.50 in helix 1 that participates in an extensive hydrogen-bonded network with Asp2.50 in TM2 also involving several structural water molecules.[36] The presence of structural water in this extensive hydrogen-bonding network, which includes residues in helices 1, 2, 6, and 7, suggests that proton transfer can occur without extensive movement of the helices.[36] Structural water is also observed in this region of the β_2-adrenergic receptor and thus is likely a feature of all GPCRs. Asn7.49 in TM7 is at approximately the same level as Asp2.50, and reciprocal mutation of these two residues in the gonadotropin-releasing hormone receptor gave a functional double mutant.[37] Asn2.40, lower in TM2, hydrogen bonds with Tyr7.53, part of an NPxxY structural motif in helix 7, discussed later.

In helix 3, Cys3.25 at the top forms a disulfide bridge with a conserved cysteine within extracellular loop 2 (EL2), observed in the structures of rhodopsin and the β_2-adrenergic receptor. In rhodopsin, Glu3.28 serves as the counterion for the Schiff's base formed between retinal and Lys7.43. In the serotonin receptors, Glu3.28 is mutated to an aromatic residue, usually Trp3.28. A more distinct role for Trp3.28 became evident in the structure of the β_2- receptor, where it is observed to be engaged in $\pi-\pi$ stacking with a tryptophan (Trp3.18) in the middle of extracellular loop 1 (EL1), seven residues back from the absolutely conserved Cys3.25. It appears that this residue also participates in an aromatic cluster that may include hydrophobic residues at positions 3.20, 3.24, and 2.60. The $\pi-\pi$ stacking between Trp3.18 in EL1 with Trp3.28 would serve to restrain EL1, help to keep it pulled down toward the helical bundle, and also help to keep the tops of helices 2 and 3 closely associated. These two tryptophans also form a wedge-shaped cleft that accommodates the disulfide bridge between Cys3.25 and the conserved cysteine in extracellular loop 2 (EL2). In addition, as noted above, this cluster may help to maintain a relatively tight association between the tops of helices 1−3.

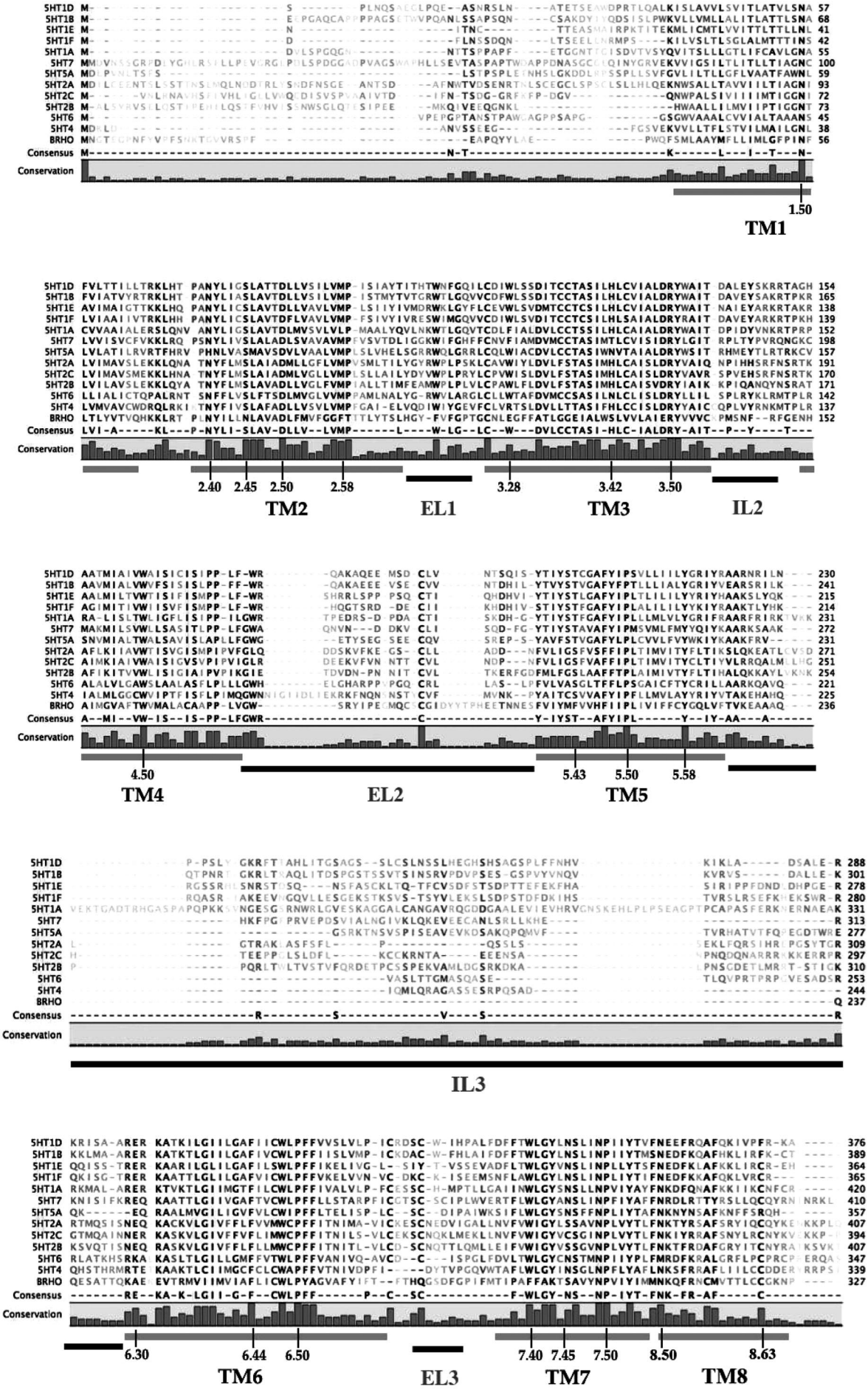


Figure 4. (1 of 2)

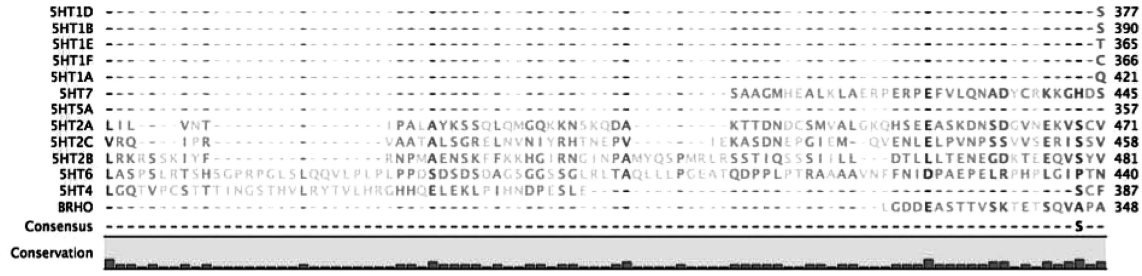


Figure 4. (2 of 2) Alignment of human serotonin receptor sequences. This figure shows an alignment of all human cloned G-protein coupled serotonin receptors compared with the sequence of bovine rhodopsin (BRHO). The consensus sequence is shown at the bottom of each segment, along with a plot showing the degree of similarity at each residue position. Absolutely conserved residues are in black, and less conserved residues are in gray. The approximate position and number of each transmembrane (TM) helix is indicated below the alignment as a gray bar. Significant residues discussed in the text are labeled according to the Ballesteros and Weinstein numbering system to designate the most conserved residue in each helix as X.50, where X is the helix number.

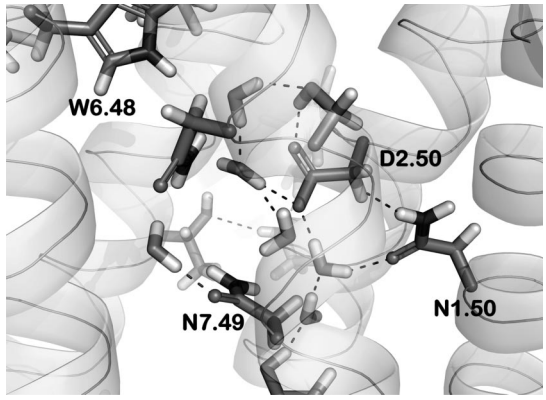


Figure 5. Illustration of absolutely conserved Asn1.50 in helix 1, showing the extensive network of hydrogen-bonded structural water that involves other conserved residues, including Asp2.50, Asn7.45, and Asn7.49 and extends toward Trp6.48.

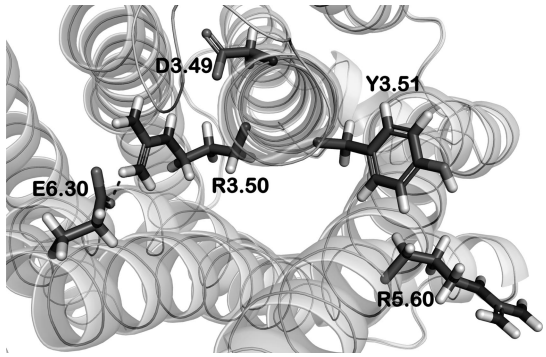


Figure 6. The conserved DRY motif at the intracellular face of helix 3, and the conserved Glu6.30 also at the bottom of helix 6 in the β_2-adrenergic receptor. The view is looking up from the intracellular space toward the bottom of the receptor bundle. It is believed that intracellular motion of helix 6 leads to disruption of a salt bridge between Arg3.50 and Glu6.30, which is thought to be a key part of the signaling mechanism.

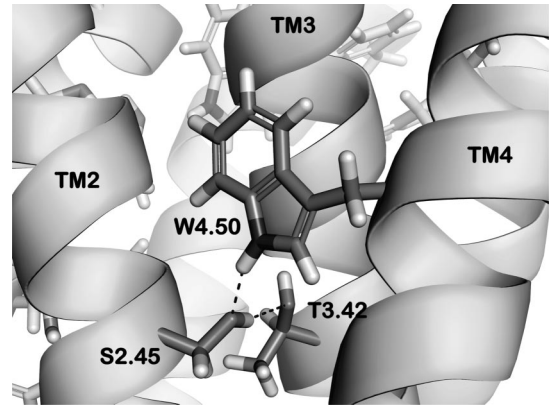


Figure 7. Absolutely conserved Trp4.50 in helix 4 hydrogen bonds to a polar residue in helix 2, which in turn can hydrogen bond to a conserved polar residue on the back of helix 3, presumably forming a hydrogen-bonded network that helps to maintain the packing of helices 2, 3, and 4.

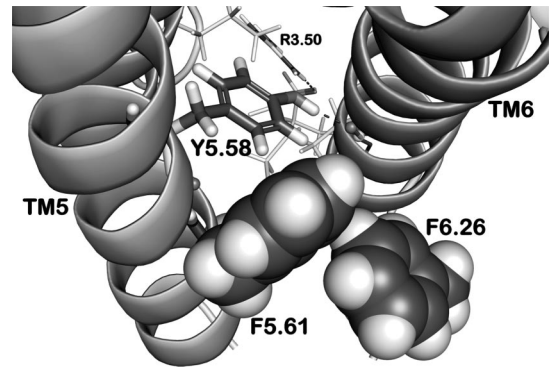


Figure 8. Conserved Tyr5.58 in helix 5 interacts with Phe5.61, which interacts with Phe6.26 near the C-terminal end of intracellular loop 3 (IL3) in the β_2-receptor. Interactions between Tyr5.58, Xaa5.61, and residues in the C-terminal region 6.24−6.26 of IL3 would affect the conformation of IL3, the portion of the receptor that is critically involved in coupling to the G-protein.

In contrast to the all of the other serotonin receptors, which have a Trp or Phe as residue 3.28, the $5HT_4$ receptor has an arginine at that location. Interestingly, only this receptor has an acidic residue at the N-terminal end of EL1, Glu3.22, suggesting the possibility of an ionic tether to Arg3.28 in this receptor subtype. Such a tether would serve the same function as the $\pi-\pi$ stacking observed for the tryptophans at these locations in the other serotonin receptor subtypes.

Also at the top of TM3, but facing outward toward the membrane, is a cluster of hydrophobic amino acids that interacts with hydrophobic residues at the tops of TM2 and TM4, and this motif may serve to anchor the tops of helices 2, 3, and 4 together. In the crystal structure of the β_2-adrenergic receptor, two cholesterol molecules also are observed in the cleft between helices 2, 3, and 4, further suggesting that these three helices may remain tightly associated and relatively stationary during receptor function. Thus, helices 1−4 may form a relatively rigid receptor core.[38]

At the bottom of TM3, all of the mammalian monoamine GPCRs have a highly conserved DRY (ERY in rhodopsin) sequence (Figure 6), located at the boundary between helix 3 and intracellular loop 2 (IL2).[39,40] Sometimes referred to as an "arginine cage", it plays a crucial role in regulating

conformational states of GPCRs. In the rhodopsin receptor inactive state, Arg3.50 is "caged" by salt bridges between Asp3.49 and Glu6.30 at the bottom of TM6. It is believed that this arginine cage constrains GPCRs in the inactive conformation.[41–43] Curiously, this salt bridge is disrupted in the published crystal structure of the β_2-adrenergic receptor, but closer examination reveals that a spurious sulfate ion has been incorporated into the crystal and formed an ionic bond with Arg3.50. Sulfate is a stronger counterion than glutamate, hence probably causing disruption of the expected salt bridge that is observed in the crystal structure of inactive rhodopsin.

Rotation of TM6 and disruption of this ionic bridge is thought to be a key part of the activation mechanism, and mutation of Glu6.30 to arginine in the 5-HT_{2A} receptor gave a mutant with constitutive activity.[44] In rhodopsin and in the 5-HT_6, 5-HT_{1D}, 5-HT_{1B}, 5-HT_{2B}, and 5-HT_{2C} receptors, a polar threonine or serine at position 6.34 also hydrogen bonds to Arg3.50 to stabilize the inactive state of the receptor.

The loop connecting the bottoms of TM3 and TM4, intracellular loop 2 (IL2), has a number of residues that appear important. A highly conserved tyrosine about five residues into the loop is in the vicinity of Glu6.30 and may be involved in stabilizing the glutamate after the salt bridge with Arg3.50 is ruptured. Two or three residues further into the loop, a polar residue, typically arginine, serves to form a salt bridge with Asp3.49. In the β_2-receptor, this residue is a serine, and it hydrogen bonds to Asp3.49. When the receptor is activated, the salt bridge between Arg3.50 and Glu6.30 is broken. Arg3.50 then collapses back toward helix 3, where it is sandwiched between Asp3.49 and a polar hydrogen bonding amino acid seven or eight residues into IL3.

Tyr3.51 is the least conserved of the triad of residues in this DRY motif, appearing in only about 74% of GPCRs, whereas Arg3.50 appears in 100% of the sequences.[45] Still, it is surprising, in view of its high degree of conservation, that no one has so far commented on the exact role of Tyr3.51. In the crystal structure of inactive rhodopsin, this residue hydrogen bonds to Gln5.60 in TM5. Residue 5.60 is an arginine in all of the 5-HT_1 family receptors, as well as in the β_2-adrenergic receptor (shown in Figure 6) but is glutamine and lysine, respectively, in the 5-HT_7 and 5-HT_{5A} receptors. Although no hydrogen bonding is observed between Tyr3.51 and Arg5.60 in the β_2-adrenergic receptor structure, the putative location on the outside of helix 5 may make it particularly labile to external polar disrupting forces that occurred during crystallization of the receptor. In the 5-HT_2 family, homology maps with the crystal structure of the β_2-adrenergic receptor suggest that Leu5.60 could interact with Tyr3.51 through van der Waals forces. Despite being highly conserved, Tyr3.51 evidently does not play a particularly important or consistent role, because mutations of this residue often had little[46] or no effect[47,48] on receptor function. In some cases, mutation of Tyr3.51 did lead to decreased cell surface expression, for example, see Auger et al.[49]

In helix 4 (Figure 7), tryptophan 4.50 is absolutely conserved. Surprisingly, there is no published discussion on the role of this conserved residue in GPCRs, but in the crystal structure of rhodopsin, it hydrogen bonds to Asn2.45 in TM2 (conserved as Ser2.45 in all of the serotonin receptors) (Figure 7). Asn2.45 also hydrogen bonds to Ser3.42 on the back of helix 3 in rhodopsin or with the polar His3.42 at that location in most of the 5-HT receptors (but as Asn3.42 in 5HT_6 and 5HT_{5A} and Thr3.42 in 5HT_7). Similarly, in the crystal structure of the β_2-adrenergic receptor, Trp4.50 hydrogen bonds to Ser2.45. A Thr3.42 residue is present in this receptor, although in the crystal structure it is about 0.8 Å too far away to interact with Ser2.45. It may be that the helical arrangement in the β_2-receptor has been altered slightly by binding to an inverse agonist. Nevertheless, it appears likely that Trp4.50 participates in a three-residue hydrogen-bonded motif with partners in helices 2 and 3, probably serving to help stabilize packing among these three helices. Once again, one sees structural motifs that serve to keep helices 1−4 associated, comprising what is likely a relatively rigid core structure.[38]

The second extracellular loop connecting helices 4 and 5 (EL2), is thought to play an important role in ligand binding. Inspection of the crystal structure of rhodopsin, as well as mutation studies, has shown that residues in the second extracellular loop are probably involved in ligand binding[50,51] or may be responsible for receptor subtype selectivity.[52,53] Amino acids in EL2 contribute to a hydrogen-bonding network that is thought to maintain rhodopsin in an inactive conformation.[54] In the rhodopsin crystal structure, EL2 appears to act as a wedge, preventing the ends of TM6 and TM7 from moving in toward the core of the protein. The importance of EL2 is also supported by the work of Patel Crocker et al.[55] showing that the C20 methyl of retinal rotates significantly toward EL2, rather than a large displacement of the ionone ring toward H3 or H6.

In the dopamine D_2 receptor, the two or three residues immediately following the conserved cysteine in EL2 probably face the binding pocket and are located near other key binding residues within the transmembrane domain.[56] Because of the length of EL2 and its putative position within the binding site crevice, it is likely that it participates with residues in the transmembrane domain in the binding of small molecule ligands and in the determination of their specificity. Mutagenesis of EL2 residues to cysteine to determine the pattern of accessibility indicates that the portion C-terminal to the conserved disulfide bond is deeper in the binding site crevice than is the N-terminal portion, a feature that is similar to EL2 in rhodopsin.[57]

Perez et al.[53] found that substitution of three consecutive residues in EL2 changed the ligand specificity for particular antagonists from that of the α_{1A} adrenergic receptor to that of the α_{1B} adrenergic receptor, and vice versa. Substitution of a single residue within EL2 of the canine and human 5-HT_{1D} receptors interconverted their specificity,[52] and in the adenosine receptor, several glutamate residues in EL2 are known to be critical for ligand recognition.[58,59] In the crystal structure of the β_2-adrenergic receptor, Cherezov et al.[30] point out that accessibility to the ligand binding site is enabled by EL2. In the M2 muscarinic receptor, Jager et al.[60] found that Trp7.35 at the extracellular top of TM7 was a contact site for residues in EL2 in the inactive receptor. These investigators showed that Trp7.35 was essential for binding of full agonists and for receptor activation by partial agonists at this receptor. In addition, Avlani et al.[61] have suggested that EL2 serves as a flexible "gatekeeper" in the binding of both allosteric and orthosteric GPCR ligands. Thus, EL2 likely contributes to the binding site in the serotonin receptors, as well as many, if not all, other type A GPCRs.

Within the GPCR type A family, residues in TM5 appear to confer ligand specificity. In rhodopsin, residues 5.42 and 5.43 are Met207 and Phe208, respectively, which likely interact with the ionone ring of retinal through van der Waals interactions, following photoisomerization of the chromophore.[62] In the 5-HT_2 family of receptors, as well as in $5HT_6$ and $5HT_4$ isoforms, Ser5.43 probably binds to the 5-OH of serotonin.[63] Residues Ser5.42 and Thr5.43 likely are involved in binding to the 5-OH of serotonin in the 5-HT_1 family,[64] as well as the 5-HT_{5A} and $5HT_7$ isoforms.

One turn lower in the helix, Phe5.47 is absolutely conserved, although not much is known about its function. The recent report by Salom et al.[62] suggests that it interacts with the agonist ligand. It also forms a stacked $\pi-\pi$ complex with Phe6.52 in TM6, a residue recognized as being crucial for agonist activation of the receptor. In rhodopsin, Phe5.47 moves upward after photoactivation, presumably following the movement of the ionone ring. In the β_2-receptor, Phe5.47 nestles against the edge of Phe6.44, forms a $\pi-\pi$ stacking interaction with Phe6.52 in helix 6, and has no contact with the inverse agonist ligand.

Lower in helix 5, Pro5.50 is considered the reference residue and introduces a kink into the helix. Molecular dynamics simulations suggest that the photoactivation of rhodopsin leads to a decreased kink angle in this helix.[65]

Tyr5.58 is actually the most conserved residue in TM5. In the β_2-receptor, Tyr5.58 is buttressed on the outside of the helical bundle by an edge-to-face $\pi-\pi$ stacking interaction with Phe5.61 and appears to hydrogen bond to the backbone carbonyl of Leu6.34 in helix 6 (Figure 8). Phe5.61 engages Phe6.26 in IL3 through an edge-to-face interaction so that movement of Tyr5.58 will be transferred to Phe6.26. In rhodopsin, Tyr5.58 appears to interact with a glutamate residue nine positions further on (5.67) within intracellular loop 3 (IL3). However, in location this residue corresponds approximately to residues 6.24 or 6.25 in the monoamine receptors, near the C-terminal portion of IL3. All of the GPCRs have polar residues in the corresponding region of IL3. A third residue at position 6.35, which is arginine in rhodopsin, either arginine or lysine in 8 of 12 of the serotonin receptors, and threonine in two others, projects outward toward residues in the C-terminal region of IL3. These observations, derived from the crystal structures, suggest that a motif comprised of residues Tyr5.58−(Tyr/Phe)5.62−6.25/6.26−6.35 interacting through hydrogen-bonding or $\pi-\pi$ stacking interactions, may be involved in stabilizing the inactive conformation of IL3, keeping it pulled upward toward helices 5 and 6.

Thus, it seems possible that upon receptor activation, when the Arg3.50/Glu6.30 salt bridge is broken, Arg3.50 collapses back toward helix 3, where it is stabilized by Asp3.49 and other local polar residues. Concomitant movement at the bottom of helix 6 then disrupts the hydrogen bond between helix 6 and Tyr5.58, allowing it to move toward residue 5.62, usually tyrosine or phenylalanine in the 5-HT receptors, which then affects the conformation of IL3 through interaction with residues in the C-terminal end of the loop. Considering the structures of both rhodopsin and the β_2-receptor, it would appear that movement of Tyr5.58 leads to structural changes that rather directly influence the conformation of IL3. Thus, one can hypothesize that Tyr5.58 is a key residue involved in inducing conformational changes in IL3. In the structure of activated rhodopsin,[62] IL3 has dropped completely away from this network into the cytoplasm, suggesting that upon receptor activation, movement of these residues may be crucially involved in producing the necessary conformational changes of IL3.

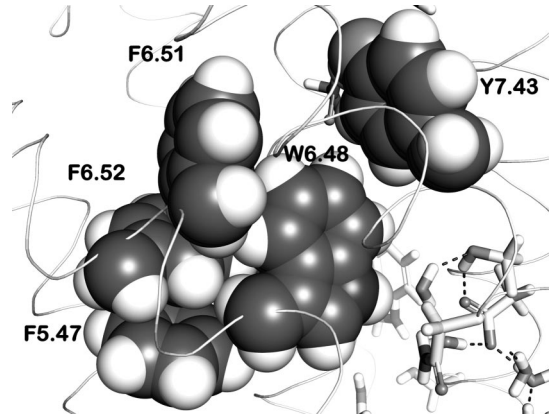


Figure 9. The aromatic cluster in helices 5, 6, and 7, sometimes referred to as the "toggle switch". Residues are shown in an inactive state, as observed in the crystal structure of the β_2-adrenergic receptor. The agonist ligand presumably interacts with one or more of these residues upon binding, displacing them from their ground state, and inducing conformational movements that transmit motion down through helices 6 and 7.

As discussed above, Glu6.30 forms a salt bridge with Arg3.50, apparently being critically involved in the receptor activation mechanism. Surprisingly, although the 5-HT_6 receptor has the conserved DRY motif at the bottom of TM3, Ala6.30 replaces the expected glutamic acid in TM6, suggesting that this receptor may have atypical properties. Indeed, the human 5-HT_6 receptor was found to have constitutive activity,[66] and the mouse receptor variant of the receptor also is reported to display strong constitutive activity.[67]

As noted earlier, Phe5.47 stacks against Phe6.52. Agonist ligand binding would presumably disrupt this interaction as well. Phe6.52 is an essential component of a cluster of aromatic residues that surrounds Trp6.48, referred to as a "toggle switch".[68] This motif includes Phe6.44 and Trp6.48 for serotonin and other monoamine receptors.

In helix 6, Trp6.48 is absolutely conserved in all GPCRs and nestles against the retinal chromophore in the inactive structure of rhodopsin. Trp6.48 forms part of an aromatic cluster that has been called a "receptor toggle switch" in TM6.[68,69] This cluster is illustrated in Figure 9, as observed in the structure of the β_2-adrenergic receptor. The movement of Trp6.48 is thought to be one of the major features of receptor activation, comprising part of a "receptor toggle switch" mechanism. A change in the conformation of Trp6.48 following the isomerization of retinal also probably leads to disruption of the extensive hydrogen-bonding network with polar residues in helices 1, 2, and 7.[36]

Phe6.52 is thought to form an edge-to-face $\pi-\pi$ interaction with the aromatic ring of agonist ligands.[70–74] In the structure of the β_2-receptor, Phe6.52 forms an edge-to-face $\pi-\pi$ aromatic interaction with the ligand, even though the ligand (carazolol) is an inverse agonist.[30] Phe6.51 is necessary for affinity of antagonists,[72–74] although a recent study has shown that Phe6.51 interacts with the *N*-benzyl substituent in a series of superpotent phenethylamine agonist ligands.[75]

In rhodopsin, the conserved Phe5.47 lays against the β-ionone ring of retinal, Phe6.44 forms part of the floor under the ionone ring, Trp6.48 lays against the ionone ring, and

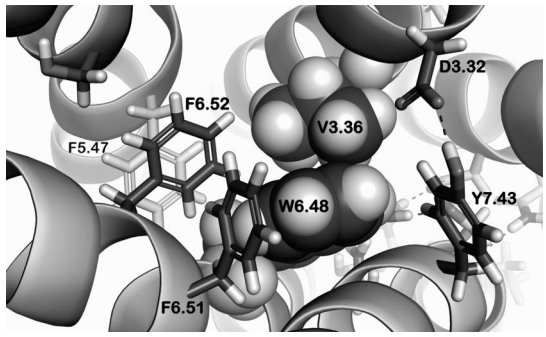


Figure 10. Extracellular view looking down into the ligand binding domain of the β_2-adrenergic receptor, showing the van der Waals association between Val3.36 and Trp6.48, and other residues of the aromatic cluster, including Phe6.51 and Phe6.52. Phe5.47 is shown in light gray below Phe6.52. Hydrogen bonding between Tyr7.43 and Asp3.32 probably helps to stabilize the receptor in the inactive state.

Ala6.52 interacts with the top of the ionone ring. When retinal photoisomerizes, the motion of the chromophore disrupts all these interactions, causing a major movement in the toggle switch motif. In the structure of light-activated rhodopsin, Phe6.44, Trp6.48, and Phe6.51 all move slightly toward helix 7, and Ala269 (6.52) is displaced downward.

Spin labeling studies suggest that light activation of rhodopsin causes helix 6 to move approximately 8 Å away from helix 3 at the intracellular surface.[76] Actual measurement of the displacement, however, by comparison of the crystal structures of inactive rhodopsin and light-activated rhodopsin, indicates a much smaller movement. The distances between the Cα carbons of Arg3.50 and Glu6.30 in the inactive molecule and the photoactivated molecule are 9.1 Å and 10.6 Å, respectively, a difference of only 1.5 Å, although the Cα carbon of Glu6.30 is displaced 2.6 Å from its location in the inactive receptor. Presumably, when a ligand binds to the serotonin receptors, it induces similar conformational changes.

It has been suggested that the activation mechanism must involve movement of the extracellular ends of helices 3, 6, and 7 toward each other.[38] If helices 1, 2, 3, and 4 are fixed relatively rigidly in the helical bundle, then the major motion would occur in helices 6 and 7. In rhodopsin, this movement has been estimated to be about 1−2 Å[77] at a level that would correspond approximately to the conserved aspartate in helix 3 of the serotonin receptors.

The structure of the β_2-adrenergic receptor provides additional important perspective on the role of Trp6.48. In the crystal structure, the position of Trp6.48 is stabilized by van der Waals interactions with Val3.36 in helix 3 (Figure 10). In the serotonin receptors, this latter residue is either serine or cysteine (or threonine in the 5-HT_4 receptor). The crystal structure of the β_2-receptor therefore indicates that one role for residue 3.36 is to stabilize the conformation of Trp6.48 in the receptor inactive state. If that hypothesis is true, then particular mutations of that residue should produce significant disruption of receptor function. Almaula et al.[78] have mutated this residue to an alanine in the 5-HT_{2A} receptor and reported that it was involved in binding primary amines of tryptamine ligands. It seems quite possible that once the hydrogen bond from Trp6.48 to Ser3.36 has been broken, Ser3.36 is then able to interact directly with the ligand.

In our own laboratory, however, the S3.36A mutant human 5-HT_{2A} receptor stably expressed in HEK cells demonstrated about a 200-fold loss of functional potency for serotonin and even more dramatic (e.g., 1000−2000-fold) potency losses for phenethylamine ligands (Braden and Nichols, unpublished). Although the intrinsic activity of serotonin was not affected in this mutant, its potency to activate inositol phosphate (IP) accumulation was reduced more than 100-fold for serotonin and more than 1000-fold for the hallucinogenic 5-HT_{2A} phenethylamine agonists 2,5-dimethoxy-4-iodoamphetamine (DOI) and 4-bromo-2,5-dimethoxy-amphetamine (DOB) (unpublished). Such severe disruptions clearly seem to indicate a fundamental role for this residue.

A highly conserved polar residue at position 6.55, which is asparagine or serine in most of the serotonin receptors, has not yet been investigated. In the β_2-adrenergic receptor, Asn6.55 is sandwiched between the ligand and helix 6. Because the ligand is an inverse agonist, it is not clear how this residue might be involved in receptor function. However, with an agonist ligand bound, simulated docking experiments in the author's laboratory suggest that it may be engaged by hydrogen bonding either to the oxygen of Ser5.43 or to the oxygen of the 5-OH of serotonin. In the β_2-receptor, Asn6.55 also can hydrogen bond to Tyr7.35, suggesting that changes in the conformation of Ser5.43 upon agonist ligand binding might be translated to helix 7 by Tyr7.35 through interactions with Asn6.55. Except for the 5-HT_{1A} receptor, all of the serotonin receptors have a polar residue at location 6.55 and most, but not all, have a complementary residue at position 7.35 or 7.36.

Extracellular loop 3 has not been studied, but one structural feature stands out that deserves comment. It will be noted from Figure 4 that, with the exception of the 5-HT_4 and 5-HT_{1E} receptors, each of these loops in the serotonin receptors contains two cysteine residues. It seems more than coincidence that this feature would be conserved unless these residues form a disulfide linkage. In the crystal structure of rhodopsin, the serine near the middle of this loop hydrogen bonds to the backbone carbonyl oxygen of the histidine residue, serving to form a short tether between these residues in the loop. In the β_2-receptor, a glutamine residue at the beginning of the loop is followed two residues later by an asparagine, and it seems possible that these residues also could hydrogen bond to form an association within the loop. It might be noted that all of the dopamine receptors, another member of the monoamine GPCR family, have two cysteine residues located within EL3. Molecular modeling studies in our laboratory have shown that a disulfide bridge between these conserved cysteines gives very reasonable conformations, and mutagenesis studies are now underway to study the role of these residues in EL3. If this structural feature is confirmed, it could mean that EL3 serves as a relatively inflexible tether between the tops of helices 6 and 7.

The first conserved residue at the top of helix 7 is Trp7.40. In rhodopsin, this residue sits behind the Lys7.43 that forms the Schiff's base linkage to retinal. In the β_2-receptor, and in the serotonin receptors and probably the other monoamine receptors, Lys7.43 has been replaced by Tyr7.43. In the β_2-receptor and probably the other monoamine GPCRs, Trp7.40 engages Tyr7.43 through an edge-to-face $\pi-\pi$ interaction. Trp7.40 is then "caged" through van der Waals interactions by a number of adjacent hydrophobic residues at the top of helices 1, 2, and 7.

Tyr7.43 is a determinant of ligand interaction in GPCRs,[31,79] but no mutation data have been reported for this residue in monoamine receptors. In the crystal structure of the β_2-adrenergic receptor, however, Tyr7.43 is observed hydrogen

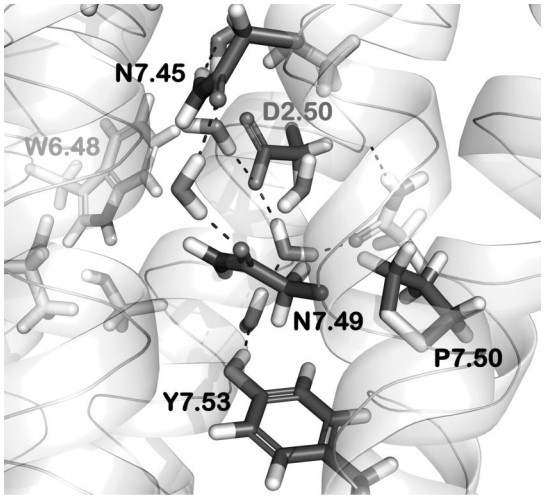


Figure 11. The "NPxxY" motif in helix 7. This motif is included in the extensive hydrogen-bonded network with structural water that resides in the core between helices 1, 2, and 7 and includes Asn1.50 and Asp2.50. Trp6.48 is shown in the background. Although the crystal structures of rhodopsin and the β_2-adrenergic receptor do not indicate that Trp6.48 is hydrogen bonding with any water molecules, there is structural water very close by, and any movement of Trp6.48 could lead to a new hydrogen-bonding scheme that would have profound consequences for the structure within this part of the receptor. For example, in the photoactivated structure of rhodopsin, Asn7.49 has rotated its amide group to hydrogen bond to Asp2.50. Unfortunately, that activated structure is only at low resolution, and the locations of structural water molecules cannot be ascertained.

bonding to the crucial Asp3.32 in TM3. The β_2-receptor was crystallized with an inverse agonist bound, which essentially means that the structure represents an inactive state. Thus, it seems likely that Tyr7.43 helps to stabilize the receptor in the unbound state. It has been proposed that residues coupled to position 7.43 comprise a linked network that extends parallel to the plasma membrane from this residue and forms the bottom of the ligand binding pocket.[32] These residues include the aromatic cluster in helix 6 that comprises the "toggle switch", discussed above. Based on the recent structure of the β_2-adrenergic receptor, Tyr7.43 and Phe6.52 are seen to be more nearly in the plane of the agonist ligand, rather than as a floor of the binding region. As noted by Suel et al.,[32] coupling from Tyr7.43 through the toggle switch region is involved in signal flow through the GPCRs from initiation of ligand binding to the final conformational state that initiates G-protein activation. Clearly, perturbation of this residue, for example, by disruption of its hydrogen bonding to Asp3.32, would have significant consequences for receptor conformation. More will be said about this idea later.

Polar residues at position 7.45 hydrogen bond to structural water that bridges to Ser7.46, and Ser7.46 hydrogen bonds to Asp2.50. These features form a sort of hydrogen-bonded cage around Asp2.50 and 7.46. Trp6.48 is directly adjacent to this motif, and when Trp6.48 is displaced by agonist binding, structural water in this region undoubtedly couples movement to these residues. In the β_2-receptor, a molecule of structural water bridges residues 7.45 and 7.49.

Located at the bottom of helix 7 is an "NPxxY" motif, comprised of Asn7.49, Pro7.50, and Tyr7.53 (Figure 11). This motif is known to provide stabilization of the receptor in its inactive state.[79–82] Asn7.49 participates in a hydrogen-bonded network, discussed earlier, which includes Asp2.50, Trp6.48, and Asn7.45, as well as with numerous structural water molecules, observed not only in the structure of rhodopsin but also in the β_2-receptor structure. In the photoactivated structure of rhodopsin, Asn7.49 has flipped its hydrogen-bonding scheme, and pulled closer to Asp2.50.

Pro7.50 introduces a kink into the helix, and the next two hydrophobic residues face outward toward the membrane. Tyr7.53 hydrogen bonds to Asn2.40 at the bottom of helix 2 and is stabilized by $\pi-\pi$ interaction with Phe8.54, near the N-terminal end of TM8. In the β_2-adrenergic receptor structure, two water molecules form a hydrogen-bonded bridge between Tyr7.53 and Asp2.50. In rhodopsin, cross-linking the double mutant Y7.53C and F8.54C prevented formation of meta II, the conformation of light-activated rhodopsin, whereas the Y7.53A or F8.54A mutations facilitated it.[83] Movement of the helices disrupts interactions of the NPxxY motif and likely leads to changes in the conformation and orientation of helix 8.

The C-terminal sequence of all the GPCRs includes a short cytoplasmic helix (helix 8) parallel to the plane of the membrane that is involved in the G-protein coupling process. Except for the 5-HT_6 receptor, all of the serotonin receptors have a conserved asparagine in the middle of the segment connecting the bottom of helix 7 and the beginning of helix 8, numbered here as 8.50. In rhodopsin, Asn8.50 is part of a polar cluster that includes Gln8.52 two residues further in helix 8, Tyr7.53, Asn2.40, and Thr2.37, and probably structural water. The only residue that has been mutated in rhodopsin is Asn2.40. For the alanine mutation, Shi et al.[84] reported a ~27% decrease in transducin activation, but the mutant was normally phosphorylated by rhodopsin kinase.[85] Cys8.63 is highly conserved, and in the β_2-adrenergic receptor, it is palmitoylated, presumably anchoring it into the membrane. The 5-HT_{1B} receptor has a cysteine two residues further on that could serve the same function, but the 5-HT_{1B}, 5-HT_{1D}, and 5-HT_{5A} receptors have a different functionality for anchoring helix 8 to the membrane in this region.

A consideration of all these structural similarities, and many others, among the serotonin receptors, the β_2-adrenergic receptor, and bovine rhodopsin is extremely compelling evidence that the overall structure and functional topography of the receptors has remained essentially the same over evolution, with external serotonin replacing the intrinsic retinal and its photoisomerism as the activating process.[31,86,87] The solution of a low-resolution (4.15 Å) crystal structure of a photoactivated rhodopsin molecule[62] has surprisingly indicated that the scale of movements in the photoactivated structure is much smaller than had been anticipated by indirect methods that had predicted large rigid body movements of the helices. This finding of small relative motion of the helices upon activation again supports the utility of GPCR homology models derived from rhodopsin. It also further suggests that relatively little energy is expended when the ligand binds and the receptor adopts an activated conformation, a feature that would seem to be necessary in a rapidly signaling switch.

Based on the foregoing discussion, a crude scenario for receptor activation can now be envisioned. Importantly, it should be remembered that the β_2 receptor structure was solved with an inverse agonist bound, essentially meaning that one is observing a receptor state that probably resembles the unliganded receptor more closely than it does an agonist bound receptor. The inverse agonist ligand molecule is longer

than a β-agonist because of the insertion of the OCH_2 unit into the side chain, a structural feature often encountered in antagonists and inverse agonists. As a result, the ligand serves as a long spacer, or "wedge", inserted between helix 5 and the conserved Asp3.32 in helix 3. All orthosteric monoamine GPCR antagonists and inverse agonists possess a basic nitrogen, usually with an aromatic ring tethered through a three to four carbon chain on one side and an aromatic system with polar functionalities extended on the other. It is not difficult to imagine that most antagonists simply form a sort of clamp over the region comprised of the aromatic residues in the toggle switch area and residues between helices 2, 3, and 7, with conserved Asp3.32 in the middle. Thus, they occupy the ligand binding domain but essentially lock the receptor into an inactive state that resembles to a certain degree the crystal structure of the β_2-adrenergic receptor.

An agonist ligand, which is *shorter* and will engage polar residues on helix 5, when placed into the ligand binding domain will exert a pull between polar specificity residues in helix 5 and the conserved aspartate in helix 3, essentially having an effect opposite to the "wedge" of an inverse agonist or antagonist. A hypothetical binding scenario follows from a consideration of the events that will occur when this shorter agonist molecule interacts within the ligand binding domain.

In the unliganded inactive receptor state, Asp3.32 in TM3 is hydrogen bonded by Tyr7.43 in TM7. The residue at position 3.36 interacts with Trp6.48, either through van der Waals forces (β_2-receptor) or by hydrogen bonding, as in the case of the serotonin receptors. Both of these key interactions help to tether helix 3 to helices 6 and 7. Aromatic residues adjacent to Trp6.48 in TM6, especially 6.44, 6.51, and 6.52, form the aromatic hydrophobic cluster that comprises the "toggle switch", and also help to stabilize Trp6.48 in its ground-state position.

When an amine ligand binds and forms an ionic salt bridge with Asp3.32, the agonist is sufficiently short in length that it causes Asp3.32 to twist toward helix 5, so that the ligand bridges between Asp3.32 in helix 3 and the polar residues in helix 5 that determine ligand specificity. As a consequence, stabilization of the position of Tyr7.43 by hydrogen bonds to Asp3.32 is lost. The steric proximity of the ligand also displaces Trp6.48, so that its interaction with residue 3.36 in TM3 is lost. Aromatic residues Phe6.51 and Phe6.52, particularly Phe6.52, then swing toward the ligand to establish $\pi-\pi$ interactions with the core aromatic ring of the ligand, moving from the position they previously occupied toward helix 7. The rearrangement of Phe6.52 also perturbs the conformation of Phe5.47. Simultaneously, the polar residue at position 6.55 swings toward helix 5 to interact with polar residue 5.43 or an oxygen atom of the ligand, or both. The combined force of these major interactions above the conserved Pro6.50 in helix 6 causes this helix to swing around the proline pivot, pulling and slightly rotating (counterclockwise, viewed extracellularly) the top of helix 6 inward toward the ligand. The shorter length of helix 6 above this proline allows a larger displacement at the more distant bottom of helix 6, and it twists and pulls away from helix 3. Glu6.30 is thus pulled away from the Arg3.50 at the bottom of TM3. This motion also causes movement at the bottom of helix 5, at the level of Tyr5.58, which disrupts interactions with residues in IL3. As noted earlier, not much actual movement must take place at the intracellular ends of helices 3 and 6, being displaced only about 1.5 Å from each other during photoactivation of rhodopsin.

At the same time, the rotation of Tyr7.43 away from Asp3.32 in TM3, as well as the movement of Trp6.48, causes conformational change within the core of helices 1, 2, and 7, proton transfer through structural water, and alterations in the extensive hydrogen-bonded network in that region, including Asn7.45 and the NPxxY motif below it, resulting in changes in the conformation at the bottom of helix 7, which affects helix 8. The net effect of these motions, the movement at the bottom of helix 6, and conformational changes in residues at the bottom of helix 5 that hold IL3 in place, is that IL3 can fall away from the ground-state conformation, where it was associated with the bottom of helices 5 and 6, and form a new conformational ensemble that includes helix 8, which disrupts coupling with the GDP-bound Gα subunit.

The structural changes that must occur within the receptor upon agonist binding are obviously innumerable. This narrative is much abbreviated, and only a somewhat speculative outline, or sketch, of a possible gross mechanism for receptor activation. Nevertheless, it is hoped that this description will be sufficiently detailed to allow the reader to visualize and gain some overall appreciation of how the receptor activation process might work. Although mutagenesis data and the recent structure for the β_2-adrenergic receptor are consistent with such an activation scenario, the exact details of the process will no doubt occupy many research laboratories for many more years to come.

1.2. Receptor Oligomerization

Like most other G-protein coupled receptors, there is now evidence that serotonin receptors form dimers in both cell culture and endogenous systems. Western blot experiments have demonstrated that 5-HT_{1B} and 5-HT_{1D} receptors, which share a high degree of homology, each form homodimers when expressed separately but form heterodimers when heterologously expressed in the same cell.[88] Fluorescence resonance energy transfer experiments with confocal microscopy, which relies upon transfer of energy from one fluorescent molecule to another to demonstrate interaction, has shown that heterologously expressed 5-HT_{2C} receptors form homodimers in live cells in culture.[89,90] Further studies have shown that the dimer binds to two molecules of ligand and that dimerization is essential for 5-HT_{2C} receptor function.[91] Other serotonin receptors for which dimerization has now been confirmed are the 5-HT_4[92] and 5-HT_{1A}[93] receptors. It seems likely that the remaining GPCR serotonin receptors also can form dimers, and GPCR oligomerization currently is a field of very active study. Additional studies suggest that the type of oligomer present can differentially influence signal transduction effector pathways. For example, whereas μ and δ opioid receptors heterodimers facilitate β-arrestin 2 signaling, destabilization of the heterodimer leads to non-β-arrestin-mediated signaling.[94] Although the physiological significance of GPCR oligomerization is not entirely understood, research in the field is rapidly growing. Nevertheless, a recent study has shown that the monomeric β_2-adrenergic receptor efficiently activates Gs and displays GTP-sensitive allosteric ligand-binding properties.[95]

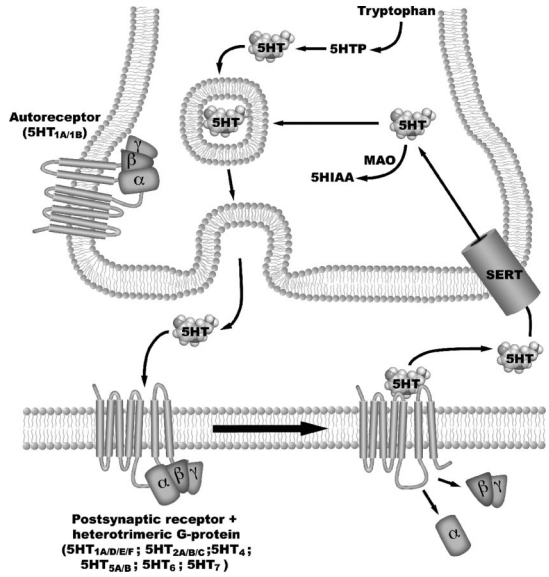


Figure 12. Model of a serotonergic synapse. Following its biosynthesis, serotonin is packaged into vesicles. When an axon potential reaches the terminal region, membrane depolarization leads to influx of calcium, and fusion of the vesicle with the terminal membrane. Serotonin is released into the synaptic space, where it diffuses across to activate postsynaptic receptors, initiating the signaling cascades within the cell. Serotonin is extracted from the synapse by specialized proteins in the presynaptic membrane, in this case the serotonin reuptake protein (SERT). The SERT pumps the free serotonin back into the neuron terminal, where it is repackaged into vesicles, to repeat the cycle. Serotonin that is free in the cytoplasm and not stored in vesicles is deaminated by monoamine oxidase in the mitochondrial membrane to produce the biologically inert metabolite 5-hydroxyindole-3-acetic acid (5-HIAA).

1.3. Receptor Activation of Heterotrimeric G-Proteins

Depolarization of serotonergic axon terminals causes an influx of calcium ions and fusion of serotonin-containing vesicles with the cell membrane (Figure 12). The serotonin is released and diffuses across the synaptic space, where it interacts with receptors located on the postsynaptic membrane. Presynaptic autoreceptors also may respond to the presence of serotonin and regulate synthesis and release within the presynaptic axon terminal. The serotonin is cleared from the synapse by a specialized reuptake protein, comprised of a bundle of 12 membrane-spanning α-helices. This transporter protein is the target for the selective serotonin reuptake inhibitor (SSRI) class of antidepressant medications like fluoxetine (Prozac) and paroxetine (Paxil). Once inside, serotonin can be repackaged into vesicles for rerelease. Monoamine oxidase located in the mitochondrial outer membrane deaminates any transmitter molecules that are not stored in vesicles.

Binding of serotonin to one of its receptors leads to activation of heterotrimeric GTP-binding proteins (G-proteins) within the cell that are coupled to the intracellular loops and C-terminus of the GPCR. These G-proteins subsequently dissociate from the receptor and interact with intracellular effectors to produce the biochemical signals that are measured following receptor activation. No attempt will be made in this review to present a comprehensive picture of current understanding of how GPCRs activate G-proteins, but a general overview will be useful. In addition to G-protein-mediated signaling, activation of GPCRs also leads to biochemical events that do not involve G-proteins.[96] Activated GPCRs can become substrates for G-protein-coupled receptor kinases (GRKs), which are then bound by β-arrestins, which inhibit G-protein interaction and lead to receptor desensitization, internalization, and activation of additional signaling pathways.

A general review of receptor-mediated activation of heterotrimeric G-proteins has recently appeared.[97] G-proteins are comprised of a Gα subunit and a dimeric G$\beta\gamma$ subunit and are grouped into four classes: $G\alpha_s$, $G\alpha_{i/o}$, $G\alpha_q$, and $G\alpha_{12/13}$.[98] In the inactive state, the receptor is coupled to a G-protein that has GDP bound within its Gα subunit. When an agonist ligand binds, the receptor undergoes a conformational change that is transmitted to the intracellular loops, in particular IL2 and IL3, which alters the coupling interactions with the G-protein. Movements of transmembrane helices 3, 5, and 6 are likely critical for this process.[99] The possible mechanism of this activation process has recently been reviewed.[97] The G-protein then undergoes conformational changes that lead to the release of GDP, the rate-limiting step in the cycle,[100] followed by binding of GTP.[101] The GTP binding then induces conformational changes in "switch regions" of the Gα subunit, followed by dissociation of the G$\beta\gamma$ dimeric subunit.[102] The activated Gα-GTP subunit and the G$\beta\gamma$ dimeric subunit can then engage a variety of enzymatic effectors within the cell. The signaling process is terminated when the GTP bound to the Gα subunit is hydrolyzed by an intrinsic GTPase. The Gα-GDP complex then reassociates with the G$\beta\gamma$ subunit, and the heterotrimer can then bind to the unliganded ground-state receptor, ready to reinitiate the signaling process when another agonist molecule binds to the receptor.

The classical view was that agonists activated receptors to produce a single signal or perhaps multiple signals but with comparable efficacy. More recently, it has become apparent that different ligands can have different degrees of efficacy in different signaling pathways. It is now believed that when agonists with different molecular structures bind to receptors, they induce and stabilize unique and distinguishable ligand−receptor conformations. These, in turn, may interact differently with downstream proteins to produce distinct patterns of signaling and ultimately cellular responses. This phenomenon has a variety of names, but a consensus term, "functional selectivity", has recently been proposed.[103–109]

2. Classification of Serotonin Receptors

The earliest classification of serotonin receptors subdivided them into two groups. The first were called "D" receptors, which generally mediated contraction of various types of smooth muscle and could be blocked by the irreversible antagonist known as dibenzyline (hence the "D").[110] The second, named the "M" receptor, mediated depolarization of cholinergic nerves and was blocked by morphine.[110] In 1979, Peroutka and Snyder[111] identified two distinct types of serotonin binding sites in brain homogenate that had high affinity for [^{3}H]LSD. One of them also had high affinity for 5-HT, and that one they named the 5-HT_1 receptor. The other site, which had high affinity for certain antagonists such as spiperone, they named the 5-HT_2 receptor. These 5-HT_2 receptors had affinity for a variety of molecules that was generally correlated with their effect at the "D" type of serotonin receptor. Both the 5-HT_1 and 5-HT_2 receptor types were eventually found to be comprised of several subtypes.

A third type of serotonin receptor that clearly differed from either the 5-HT_1 or 5-HT_2 receptors was then identified and named the 5-HT_3 receptor.[112] This receptor, a ligand-gated ion channel, has a very different molecular structure and signaling mechanism from the other subtypes, which are all G-protein coupled receptors.

The initial receptor classification systems were based on differential radioligand affinity and specific functional assays, typically contraction or relaxation of various types of smooth tissues. Among the possible ways to classify all of the presently known serotonin receptors, we believe that grouping them according to their primary signaling mechanism may be the most useful. Thus, in this review, we group the receptors into families depending on whether they primarily signal by coupling to $G\alpha_q$, $G\alpha_{i/o}$, or $G\alpha_s$ G-proteins. Further, although receptors may have been previously characterized by coupling to only one type of G-protein, much recent work has shown that GPCRs can couple not only to more than one G-protein but to a variety of other types of intracellular signaling molecules.[103–107,109]

2.1. $G_{q/11}$-Coupled Receptor Types

$G\alpha_q$ coupled receptors lead to the hydrolysis of membrane phosphoinositides, resulting in the formation of diacyl glycerol (DAG) and inositol phosphates, which then act as signaling molecules to activate, for example, protein kinase C (PKC) and elevate intracellular calcium, respectively. Another major function of the Gαq family, which includes $G\alpha_{12/13}$, is to regulate structural changes within the cell. These are primarily accomplished through activation of the Rho signaling pathway, which induces stress fiber formation and focal adhesions. Accordingly, as discussed later, the $G\alpha_q$-coupled 5-HT_2 family of receptors is significantly involved in both developmental and cell migration processes, likely through these mechanisms.[113]

2.1.1. The 5-HT_{2A} Receptor

The human 5-HT_{2A} receptor was first cloned by Branchek et al. in 1990.[114] This receptor has particular interest because of its role in normal brain function. The powerful psychoactive substances commonly known as psychedelics (hallucinogens such as LSD) presumably have 5-HT_{2A} receptors as their primary target.[115]

The first autoradiography studies to map 5-HT_2 binding sites in rat brain identified areas with high receptor density in the claustrum, with very high labeling in all areas and layers of the neocortex.[116] In the cortex, the highest binding density was localized to a continuous band that included layer IV and extended into layer III, depending on the area studied. A PET study in humans using N(1)-([^{11}C]-methyl)-2-bromoLSD found highest binding in the frontal and temporal cortices, with lower levels in the parietal cortex and motor regions, intermediate levels in basal ganglia, but only very low levels in thalamus.[117] In the thalamus, the 5-HT_{2A} receptor is expressed primarily in sensory and nonspecific nuclei.[118]

High-density 5-HT_2 binding sites in neocortex were later specifically identified as 5-HT_{2A} receptors, and that identity has been confirmed by many later studies. This localization is consistent with the observation of a dense band of 5-HT_2 receptors in upper layer V that is in register with a dense plexus of fine 5-HT axons.[119]

Pazos et al.[120] subsequently examined anatomical distribution of 5-HT_2 receptors in human brain with light microscopic autoradiography using the antagonist ligand [^{3}H]ketanserin, which is fairly selective for human 5-HT_{2A} receptors. 5-HT_{2A} receptor densities were heterogeneously distributed, with very high concentrations localized over layers III and V in several cortical areas, including the frontal, parietal, temporal, and occipital lobes, the anterogenual cortex, and the entorhinal area.

McKenna and Saavedra carried out autoradiography studies in rat brain using the nonselective 5-$HT_{2A/2C}$ agonist *R*-(−)-[^{125}I]-DOI.[121] Areas with highest binding were the claustrum and the frontal cortex. Lower expression was seen in the caudate, nucleus accumbens, and olfactory tubercle. Several autoradiographic and *in situ* hybridization studies have observed high densities of 5-HT_{2A} receptors and transcripts in the cortex,[119,122–124] and an *in situ* hybridization study of human cortex demonstrated that 5-HT_{2A} receptor mRNA was expressed on both pyramidal and nonpyramidal cells.[125]

Willins et al.[126] reported dense labeling of apical dendrites of pyramidal cells, with a small amount of labeling on cortical interneurons. Synaptic 5-HT terminals always made asymmetrical junctions that were exclusively located on dendritic spines and shafts, appearing more frequently on spines in the deep frontal and the upper occipital cortex.[127]

Higher resolution localization studies of 5-HT_{2A} receptor in primate (*Macaca mulatta*) brain by Jakab and Goldman-Rakic[128] observed expression in the cortical sheet, with weak staining in layer IV, but flanked by two intensely labeled bands in layers II and III and layers V and VI. They noted the appearance of 5-HT_{2A} receptors on virtually all pyramidal cells, with the label consistently seen on the apical dendrites, most intensely in the proximal part of the dendrite. Dendritic spines were rarely or weakly labeled, a finding consistent with studies in rat and monkey prefrontal cortex[118,129] but somewhat at odds with the report of Seguela et al.[127] In addition, Jakab and Goldman-Rakic[128] identified presynaptic 5-HT_{2A} receptors in a minor group of asymmetric synapse-forming cortical axons and suggested that 5-HT_{2A} receptors may presynaptically modulate excitatory neurotransmission in a discrete cortical axonal system. In cortical interneurons, 5-HT_{2A} receptors were expressed on large and medium size interneurons, whereas no labeling was observed on small or medium size interneurons.

Miner et al.[130] employed immunoperoxidase labeling to determine the localization of 5-HT_{2A} receptors in the middle layers of the rat prefrontal cortex. Most 5-HT_{2A} receptors were located within postsynaptic structures, predominantly on proximal and distal dendritic shafts, on both pyramidal and local circuit neurons. Most commonly, 5-HT_{2A} receptors were restricted to a particular area of the dendrite, usually extrasynaptic regions apposed to unlabeled dendrites. Of the immunopositive sites, 73% were postsynaptic, of which 58% were on dendritic shafts and 42% were present in dendritic spines. This study provided the first evidence of extensive localization of 5-HT_{2A} receptors to the heads and necks of dendritic spines. These results are consistent with those of Seguela et al.,[127] who found that synaptic serotonin terminals always made asymmetric junctions that were exclusively found on dendritic spines and shafts and appeared more frequently on spines than shafts in the deep frontal and the upper occipital cortex.

The observation that 5-HT_{2A} receptors were localized to extrasynaptic portions of dendritic shafts led Miner et al.[130] to suggest that the actions of serotonin within the cortex may be mediated at least partially through volume transmission mechanisms. This hypothesis is consistent with findings from other immunocytochemical studies, where 5-HT_{2A} receptor labeling was located some distance from 5-HT terminals in other regions of rat cortex.[131] Seguela et al.[127] estimated that only about 38% of serotonin axons in cortex engage in synaptic contact. In addition, in four different cortical areas, dendritic shafts and spines and other axonal varicosities were often seen in the immediate microenvironment of immunostained varicosities. Thus, a view has developed of a highly divergent serotonin afferent system likely capable of widespread, global, and sustained influence in the cortex.

Short-term agonist exposure of 5-HT_{2A} receptors leads to desensitization-mediated phosphoinositide hydrolysis in several transfected cell systems.[132–134] Interestingly, in contrast to most other G-protein coupled receptors, the 5-HT_{2A} receptor undergoes down-regulation in response either to agonist or antagonist treatment.[135] Recently, two nonconserved residues in the 5-HT_{2A} receptor, Ser421 in the C-terminus and Ser188 in intracellular loop 2, have been found to be essential for the agonist-induced desensitization process in cloned receptors expressed in HEK-293 cells.[136] Mutation of either residue to alanine dramatically attenuated 5-HT-mediated desensitization. Furthermore, there is a single nucleotide polymorphism (SNP), H452Y, that occurs at a frequency of 9% in the general population. When the H452Y form of the receptor is heterologously expressed in NIH3T3 cells the desensitization phenotype is dramatically altered.[137] In humans, this SNP has been associated with attention deficit hyperactivity disorder,[138] response to atypical antipsychotic medications,[139] and, interestingly, a significant reduction in temporal lobe white matter in the brain.[140]

At the present time, there seems to be a fairly clear consensus that the key site for hallucinogen action is the 5-HT_{2A} receptor subtype.[114,121,141–152] This conclusion was initially developed by correlation of the rat behavioral activity of hallucinogenic amphetamines with their affinities and efficacies at the 5-HT_2 receptor.[153–156] Perhaps the most definitive proof was the report by Vollenweider et al.[157] that the hallucinogenic effect of psilocybin in healthy human volunteers was blocked by the antagonist ketanserin, which is relatively 5-HT_{2A}-selective in humans, as well as the 5-HT_2 antagonist ritanserin.

What is the normal role for this receptor in the cortex? At the membrane level, activation of 5-HT_{2A} receptors leads to membrane depolarization and the closing of potassium channels in several brain areas, including piriform cortex[158] and neocortex.[159,160] In most layer V pyramidal cells of the medial prefrontal cortex (mPFC), focal application of 5-HT to apical dendrites induces excitatory postsynaptic currents (EPSCs) mediated by 5-HT_{2A} receptors, with the most pronounced effect being an increased frequency of EPSCs.[161] Local application of the hallucinogenic phenethylamine 5-$HT_{2A/2C}$ agonist DOI enhances late EPSCs evoked by electrical stimulation of afferent fibers.[162]

The somatosensory cortex displays a strong *c-fos* response to acute DOI challenge[163] and also receives glutamatergic projections from the ventrobasal thalamus. DOI induces *c-fos* expression in a band of neurons spanning superficial layer V to deep layer III, an area in register with the apical dendritic fields of cortical pyramidal cells.[141,163,164] The vast majority of *c-fos*-positive cells in the somatosensory cortex do not express 5-HT_{2A}-like immunoreactivity,[141,164] suggesting that the DOI-induced increase is indirectly mediated. Furthermore, *c-fos*-expressing neurons were more concentrated in septa than in barrels, suggesting that DOI activated intercortical projections. The enhanced *c-fos* expression was blocked by the selective 5-HT_{2A} antagonist M100907, but not by a 5-HT_{2C} antagonist.[141] LSD, acting through 5-HT_{2A} receptors, also produces a robust activation of the neuronal activity marker *c-fos* in the prefrontal cortex, particularly in anterior cingulate and medial prefrontal cortex.[165] As is the case with DOI, double-labeling immunohistochemistry experiments show that LSD-induced *c-fos* immunoreactivity is not present in cortical cells expressing 5-HT_{2A} receptors, further supporting the idea of an indirect activation of cortical neurons.[165] In addition to elevating *c-fos* expression within the brain, LSD activation of 5-HT_{2A} receptors in the prefrontal cortex of rats induces a dynamic pattern of gene expression changes. Significantly, many of these genes have been shown to participate in mechanisms of synaptic plasticity.[166–168]

In the periphery, 5-HT_{2A} receptors are expressed in several cardiovascular related tissues, where they modulate a variety of functions.[169,170] These include proliferation of arterial fibroblasts through p38MAPK,[171] migration of aortic smooth muscle cells through Rho-kinase and extracellular signal-regulated kinase (ERK) pathways,[172] and arterial vasoconstriction by mechanisms that may involve inhibiting Kv1.5 currents through phospholipase C (PLC) and PKC activity.[173] 5-HT_{2A} receptors also are found in C-fibers and dorsal root ganglia of the spinal cord, where their activation produces analgesia, and blockade produces hyperalgesia.[174–176]

2.1.2. The 5-HT_{2B} Receptor

The 5-HT_{2B} receptor was initially cloned from RNA isolated from rat stomach fundus using degenerate PCR primer techniques and was called the 5-HT_{2F} receptor.[177] The 5-HT_{2B} receptor has the distinction of being the only serotonin receptor necessary for viability, as knockouts of this gene in mice are lethal and produce severe embryonic defects.[178] In the adult mammal, this receptor is expressed at the highest levels in the liver, kidneys, stomach fundus, and gut, with moderate expression in the lung and cardiovascular tissues. Only weak, limited expression is observed in the brain.[179–183] This expression, however, has been linked to certain behaviors, including association with vulnerability to drug abuse.[184] Interestingly, this receptor also is associated with proper function of the auditory system, is expressed in both the cochlea and inferior colliculus, and may be involved in age-related hearing loss.[185]

Arguably, the most important function of this receptor is during development, where it coordinates the proper formation of key structures such as the heart and brain.[178,186,187] In developing cardiomyocytes, serotonin, through 5-HT_{2B} receptors, signals through $G\alpha_q$, phospoinositide 3-kinase (PI3K)/AKT, and ERK 1/2 pathways and activates NF-κB to regulate the mitochondrial adenine nucleotide translocator and cell survival and proliferation.[188,189] One of the more infamous roles of the 5-HT_{2B} receptor lies in its function in the adult heart, where its activation can lead to myofibroblast proliferation and valvular heart disease, as was the likely mechanism leading to the sometimes fatal pathologies observed in the hearts of patients taking the appetite

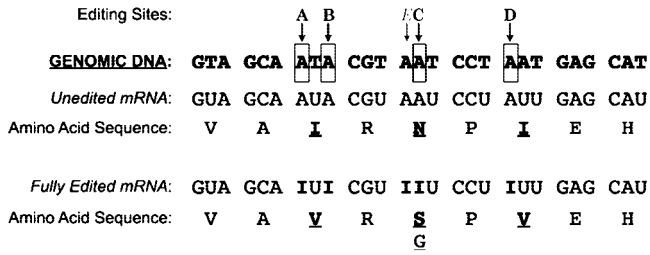


Figure 13. RNA editing of the 5-HT_{2C} receptor. ADAR mediated deamination of adenosine to inosine occurs within the region coding the second intracellular loop. There are four editing sites in rodents (A−D) and an additional editing site in humans (E). Editing results in 32 different expressed mRNA variants and 24 protein variants. In general, editing serves to silence constitutive activity through reduced coupling to Gα proteins.

suppressant combination "fen-phen".[190,191] Both fenfluramine and its active metabolite norfenfluramine are 5-HT_{2B} receptor agonists.[192] Whereas agonists of this receptor can produce valvulopathies and other cardiopulmonary problems, antagonists of this receptor may prove useful for treating diseases such as cardiac hypertrophy.[193]

2.1.3. The 5-HT_{2C} Receptor

The 5-HT_{2C} receptor was one of the first serotonin receptors cloned[194] and was initially named the 5-HT_{1C} receptor. After additional serotonin receptors were identified and found to belong to distinct families based upon G-protein coupling and sequence homology, this receptor was reclassified as the 5-HT_{2C} receptor.[195] It shares significant identity with other members of the family, the 5-HT_{2A} and 5-HT_{2B} receptors, and because of that, there are few isoform - selective antagonists and as yet no truly selective agonists. This receptor is highly expressed and was first identified in the choroid plexus, where it may serve to regulate ion exchange between the brain and the cerebrospinal fluid. Receptor mRNA and protein also are found widely distributed throughout the brain, including the cortex, amygdala, basal ganglia, hippocampus, and thalamus.[196,197]

There are three known splice isoforms of the 5-HT_{2C} receptor that include the transcript encoding for the full-length protein, as well as two splice isoforms that give rise to two dramatically truncated proteins.[198,199] The truncated isoforms are thought to be inactive and are expressed together with the full-length protein. Comparison of the genomic sequence with cloned cDNAs revealed that the primary transcript undergoes RNA editing.[200] In this process, enzymes called "adenosine deaminases acting on RNA" (ADARs) are involved. ADAR1 and ADAR2 deaminate specific adenosines in the 5-HT_{2C} receptor mRNA to form inosine.[200] Inosine is read by the transcription machinery as guanosine, which can result in amino acid substitutions in the receptor protein. There are four editing sites within the region coding for the second intracellular loop in rodent mRNA and an additional fifth editing site in humans, which together can produce 32 different mRNAs and 24 different proteins (Figure 13). In general, the level of constitutive activity of the receptor decreases with the degree of editing.[201] Other functional consequences of editing are a loss of the high-affinity agonist binding state,[202] as well as a delay in agonist-stimulated calcium release in the fully edited isoforms.[203] Furthermore, although the fully edited isoform couples to both $G_{q/11}$ and G_{13}, editing reduces or eliminates coupling to G_{13} and subsequent signal transduction pathway activation.[204,205] Together, these results demonstrate that editing serves to silence constitutive activity through reduced coupling to Gα proteins. There has been significant work to elucidate clinical relevance of 5-HT_{2C} receptor editing. These studies have suggested links between editing profiles and depressed suicide victims,[206,207] schizophrenia,[208] anxiety,[209] depression,[210] and spatial memory.[211] These links are, however, tenuous, and more work needs to be done to strengthen them.

Within the brain, 5-HT_{2C} receptors have been shown to modulate mesolimbic dopaminergic function, where they exert a tonic inhibitory influence over dopamine neurotransmission.[212–214] Because of this activity, there is considerable interest in this receptor as a therapeutic target for treating psychostimulant abuse.[215,216] The 5-HT_{2C} receptor also is believed to play roles in the mechanism of action of the SSRI class of antidepressants,[217] as well as some atypical antipsychotic medications.[218,219] There is a high level of expression of the 5-HT_{2C} receptor in the amygdala, a brain structure that mediates anxiety. Recent studies using fMRI have demonstrated that 5-HT_{2C} receptor agonists produce neuronal activation of the amygdala that is correlated with anxiety states.[220] Accordingly, selective antagonists of this receptor may have therapeutic potential as anxiolytic drugs.[221,222] A proposed mechanism for how the 5-HT_{2C} receptor modulates anxiety involves regulation of corticotropin-releasing hormone in response to stress.[223]

An additional area of considerable interest is the role of the 5-HT_{2C} receptor in body weight regulation and obesity. These receptors are expressed in many brain regions involved in regulating food intake, which include the nucleus of the solitary tract, dorsomedial hypothalamus, and the paraventricular hypothalamic nucleus.[224] In addition to experiencing sometimes fatal seizures, the 5-HT_{2C} receptor knockout mouse is severely obese and is defective in food intake regulation.[225,226] Furthermore, pharmacological manipulation of 5-HT_{2C} receptor activity also can influence appetite. Antagonists and inverse agonists of the receptor can produce significant weight gain in both rodents and humans,[227,228] whereas agonists may have a therapeutic benefit as antiobesity agents.[229,230]

2.2. G_s-Coupled Receptor Types

Activation of $G\alpha_s$-coupled receptors leads to stimulation of adenylyl cyclases, resulting in the conversion of ATP to cyclic AMP (cAMP). Cyclic AMP is a ubiquitous intracellular messenger that interacts with numerous targets, including cyclic nucleotide-gated ion channels and the phosphorylating enzyme protein kinase A (PKA). These effectors regulate calcium ion flux and membrane excitability, as well as a variety of other cellular processes. PKA also phosphorylates cAMP-responsive transcription factors, such as the cAMP response element binding protein (CREB), which leads to changes in gene expression.

Originally, it was believed that the cellular effects of cAMP were mediated exclusively by PKA. More recently, however, evidence has been accumulating that an Epac family of cAMP sensors plays an important role. Epac is an acronym for exchange proteins directly activated by cAMP, a family of cAMP-regulated guanine nucleotide exchange factors that mediate PKA-independent signal transduction. Binding of cAMP to Epac leads to the activation of Rap and Ras GTPases, as well as a number of other important

cellular proteins. The recent review by Holz et al.[231] provides an excellent overview of the role of Epac in cellular function.

2.2.1. The 5-HT$_4$ Receptor

The 5-HT$_4$ receptor was cloned in 1995,[232] is expressed both centrally and peripherally, and has at least nine splice variants. In humans, the 5-HT$_4$ receptor is expressed in the CNS in the basal ganglia, cortex, hippocampus, and substantia nigra.[233,234] Because of its strong expression in the hippocampus and coupling to stimulation of cAMP and PKA, numerous electrophysiological studies have been performed to examine its function in long-term potentiation (LTP) and synaptic plasticity. These experiments have shown that 5-HT$_4$ receptors mediate inhibition of calcium-activated potassium currents responsible for slow after-hyperpolarization in pyramidal cells in the CA1 region of the hippocampus, resulting in these cells being better able to respond to excitatory inputs.[235,236] Furthermore, receptor activation appears to influence only long-term depression (LTD) and not LTP in the CA1 region.[237] Studies in the 5-HT$_4$ receptor knockout mouse indicate that 5-HT$_4$ receptors mediate a tonic and positive influence on activity of serotonin neurons in the dorsal root ganglia and modulate 5-HT content in the raphe nuclei.[238] Behaviorally, 5-HT$_4$ specific agonists can effectively enhance learning and memory in animal models.[239–242] Further function for this receptor has been elucidated using the 5-HT$_4$ receptor knockout mouse, which displays normal behaviors under standard environmental conditions but shows abnormally low locomotor activity and hypophagia in response to novelty and stress.[243] The knockout mouse also demonstrates increased sensitivity to the convulsant pentylenetetrazol, which may be related to 5-HT$_4$ receptor expression on γ-aminobutyric acid (GABA)ergic neurons.[243]

The 5-HT$_4$receptor plays a significant role in the periphery, especially in gastrointestinal function. The receptor is expressed in both enterochromaffin cells and enteric neurons. In enteric nerve and muscle, 5-HT$_4$ stimulation facilitates acetylcholine release and relaxation of the colon.[244,245] Accordingly, therapeutics that are agonists of this receptor have been used in the clinic to treat both constipation and constipation-predominant irritable bowel syndrome (IBS).[246,247] Recently, however, the most widely prescribed therapeutic for these conditions, Tegaserod (Zelnorm), was withdrawn from the market in a number of countries due to increases in potentially fatal cardiovascular events, in particular heart attacks and strokes, observed in clinical trials. Although the molecular basis of these adverse events is currently unknown, they could result from activation of 5-HT$_4$ receptors expressed in the heart, which may modulate calcium currents.[248,249] Conversely, development of 5-HT$_4$ antagonists may have potential for treating cardiac arrhythmias.[250]

2.2.2. The 5-HT$_6$ Receptor

The 5-HT$_6$ receptor was cloned in 1993[251–253] and is expressed almost exclusively within the mammalian CNS. The highest levels of expression are found in the striatum, nucleus accumbens, cortex, and olfactory tubercle. Expression also is seen in the hippocampus, thalamus, amygdala, hypothalamus, and cerebellum.[254–256] Expression is postsynaptic, with little to no expression on cell bodies.[254] Because 5-HT$_6$ receptor expression is restricted to the CNS and there are high levels of expression detected in cortical areas, a role for this receptor in higher order cognitive processes has been hypothesized. Until the recent development of selective ligands, however, the role of this receptor has remained elusive. The authors of a recent study of 45 selective ligands have proposed a pharmacophore model for 5-HT$_6$ receptor antagonists.[257]

Although there are many selective antagonists for the 5-HT$_6$ receptor, as is the case with most GPCRs, there are only a handful of selective agonists.[258] These ligands have proven crucial in defining a function for this receptor, which appears to modulate a wide variety of neurotransmitters. Blockade of the 5-HT$_6$ receptor enhances cholinergic neurotransmission and has been shown in animal models to facilitate learning and memory processes.[259–262] Interestingly, however, the 5-HT$_6$ receptor is not expressed on cholinergic neurons.[263] Glutamate levels also are influenced by 5-HT$_6$ receptor activity: antagonists can increase glutamate release in cortex,[264] and agonists can attenuate glutamate release in hippocampal slice culture.[265] It also has been demonstrated that drugs acting at 5-HT$_6$ receptors can alter dopamine levels, likely through indirect means, as well as GABA and norepinepherine levels, although how the receptor modulates each of these neurotransmitters remains unclear.[266] Nevertheless, these effects on neurotransmission and behavior have made the receptor an attractive target for potential cognitive enhancement and in the treatment of cognitive deficits in Alzheimer's disease and schizophrenia.[266] Novelty-seeking behavior in rats also has been reliably linked to higher levels of 5-HT$_6$ gene expression in the olfactory tubercle.[267]

Recent work suggests that this receptor also may play a role in eliciting a component of the antidepressant effects of selective serotonin reuptake inhibitor antidepressants. Whereas 5-HT$_6$ receptor antagonists block the effects of SSRIs, agonists may have antidepressant-like effects, at least in animal models.[268,269] Another promising role for the 5-HT$_6$ receptor as a therapeutic target is in obesity, where both molecular and pharmacological approaches demonstrate 5-HT$_6$ receptor involvement.[270]

2.2.3. The 5-HT$_7$ Receptor

This receptor was cloned independently in 1993 by three different groups.[271–273] Interestingly, one of the cloning methods involved screening mammalian cDNA libraries at reduced stringency with probe generated from the Gα_s coupled *Drosophila* 5-HTdro1 receptor (later renamed 5-HT$_7$Dro).[273] These groups used Northern blots and in situ hybridization to demonstrate 5-HT$_7$ mRNA in the hypothalamus, thalamus, and hippocampus, as well as in the cortex. The expression of the receptor protein generally correlates well with distribution of the mRNA, where the pyramidal cell layer of all three CA regions of the hippocampus shows immunoreactivity for the 5-HT$_7$ receptor,[274,275] with slightly higher expression in CA3 and lower density in CA1, based on several studies in knockout mice.[276,277] The 5-HT$_7$ receptor also is expressed in peripheral blood vessels, where it produces smooth muscle relaxation, and in the circular smooth muscle of the human colon, although no functional effects on muscle relaxation have been detected in the latter tissue.[245]

There is a relative paucity of available data on the 5-HT$_7$ receptor and its functions, once again due in large part to the lack of selective agonists specific for this receptor isoform. Unfortunately, agonists for the 5-HT$_7$ receptor also have high agonist activity at the 5-HT$_{1A}$ receptor and affinity

at α_{2A} receptors.[276] Nevertheless, the 5-HT_7 receptor is believed to be important in regulating sleep, circadian rhythms, and the overall mood of an individual.[278]

The availability of relatively selective 5-HT_7 *antagonists* has, however, allowed studies that demonstrate the role of this receptor in regulating body temperature. 5-HT_7 antagonists block 5-HT-induced hypothermia in guinea pigs, and in rats, 5-HT_7 antagonists increase the time to onset of REM sleep as well as reducing the time spent in REM.[279,280] The role of the 5-HT_7 receptor in thermoregulation has been confirmed in 5-HT_7 receptor knockout mice, where 5-HT or 5-HT_7 receptor agonists fail to produce hypothermia.[281,282]

One of the most intriguing areas of potential 5-HT_7 receptor involvement is in depression. The 5-HT_7 knockout mouse shows reduced immobility in the forced swim test, compared with wild-type controls. The reduction was similar to the effect of an antidepressant, demonstrating an "antidepressant-like" phenotype.[283] Perhaps not surprisingly, therefore, 5-HT_7 receptor antagonists facilitated the antiimmobility effect of antidepressants in mice.[284] In vivo studies have found that both tricyclic and SSRI-type antidepressants induce *c-fos* expression in the suprachiasmatic nucleus of the rat.[285]

5-HT_7 receptor mRNA is expressed in the superchiasmatic nucleus (SCN), where evidence points to its involvement in circadian rhythms and sleep. Application of 8-OH-DPAT to hamster SCN slice cultures, in the presence of WAY100635 to block 5-HT_{1A} receptor activation, produces phase advances in neuronal firing.[286] Additionally, 5-HT_7 receptors in the dorsal and median raphe nuclei appear to modulate circadian processes by regulating 5-HT release in the hamster SCN.[287] Studies in the knockout mouse also suggest a role for 5-HT_7 receptors in circadian processes, however, there are significant differences between mouse and hamster.[288]

2.3. $G_{i/o}$-Coupled Receptor Types

Activation of this class of receptor leads to inhibition of adenylyl cyclase and decreased production of cAMP as the primary functional end point. Functional assays typically involve treating the cells expressing these receptors with forskolin, a diterpene obtained from the Indian coleus plant that stimulates adenylyl cyclase. In cells expressing a $G_{i/o}$-coupled receptor, application of serotonin or an agonist ligand for that receptor type will functionally antagonize the effect of forskolin, that is, attenuating the cAMP increase observed following forskolin stimulation. The function of these receptors also can be blocked with pertussis toxin, which prevents the dissociation of the Gα and G$\beta\gamma$ subunits.[289,290] The most commonly observed functional end point of $G\alpha_i$-coupled receptors in neurons is membrane hyperpolarization, leading to an inhibition of neuronal firing.

2.3.1. The 5-HT_{1A} Receptor

The 5-HT_{1A} receptor was the very first of the many serotonin receptors to be cloned and characterized, and a large volume of literature has been published on its physiological function and role as a potential drug target. Like all 5-HT_1-type receptors, the 5-HT_{1A} receptor is characterized by its high affinity for 5-HT. Kobilka et al.[291] first described a genomic clone, identified as G-21, which was isolated from a library using a low-stringency full-length β_2-adrenergic receptor hybridization probe. They noted that this intronless clone had a high degree of resemblance to adrenergic receptors and proposed that it coded for a GPCR. Subsequently, Fargin et al.[25] reported that the protein product of G-21, when transiently expressed in monkey kidney cells, had ligand-binding characteristics of the 5-hydroxytryptamine 1A (5-HT_{1A}) receptor. The mRNA for this receptor is found in the brain, spleen, and neonatal kidney.[291–293]

5-HT_{1A} receptors are located both pre- and postsynaptically within the brain, and at either location, their activation leads to neuronal hyperpolarization and reduced firing rate. In the brainstem, where they are expressed on cells in the dorsal and median raphe nuclei, they act as somatodendritic autoreceptors to inhibit cell firing. These cells send ascending serotonin fibers to all parts of the forebrain, and decreases in their rate of firing therefore attenuate serotonin biosynthesis and release throughout these projection areas in the brain. The presynaptic 5-HT_{1A} receptors expressed on raphe cells couple to $G\alpha_{i/o}$ proteins that activate inwardly rectifying potassium channels (GIRKs), causing neuronal membrane hyperpolarization,[294] which leads to a decreased rate of cell firing.

Postsynaptic 5-HT_{1A} receptors are expressed at high density in limbic areas of the brain such as the hippocampus and septum and in the entorhinal cortex.[295] In the hippocampus, they are highly expressed in the CA1 and CA2 fields and dentate gyrus.[296] They also are expressed at high density in layers II and VI in the frontal cortex, with lesser expression in other layers.[295] In the cortex, they are found on the axon hillock of pyramidal cells, where their activation hyperpolarizes the cell membrane. Amargos-Bosch et al.[297] reported that approximately 60% of prefrontal cortical neurons in rat and mouse express 5-HT_{1A} and 5-HT_{2A} receptor mRNAs, which are highly colocalized. Microdialysis experiments have shown that the increase in local 5-HT release evoked by the activation of 5-HT_{2A} receptors in mPFC by a 5-$HT_{2A/2C}$ receptor agonist was reversed by coperfusion of 5-HT_{1A} agonists. This inhibitory effect of 5-HT_{1A} agonists was absent in mice lacking 5-HT_{1A} receptors.[297]

Extensive research on the 5-HT_{1A} receptor has been carried out because of its possible role in anxiety. Azapirone-type drugs (e.g., buspirone), which act as 5-HT_{1A} receptor agonists and partial agonists,[298,299] have been developed as novel anxiolytic agents that are not associated with the dependence and side effect profile of the benzodiazepines. 5-HT_{1A} receptor knockout mice have been used as genetic models of anxiety and show increased responsiveness to stress.[300] They display a range of behaviors indicating elevated levels of anxiety,[301] tend to avoid novel or fearful environments, and escape from stressful situations.[302] Interestingly, the anxiety-related effects seen in the 5-HT_{1A} receptor knockout mouse are due to developmental defects. Conditional knockout mice with receptor expression restored in the hippocampus and cortex during development, followed by gene inactivation in the adult, appear behaviorally normal.[303] Conditional expression in the raphe nuclei during development, however, does not rescue the anxiety phenotype of the knockout.[303] 5-HT_{1A} knockout mice also show an attenuated hypothermic response following administration of 5-HT_{1A} receptor agonists,[301] an effect believed to be mediated by 5-HT_{1A} receptors in the hypothalamus.[304] Systemic administration of a 5-HT_{1A} agonist also induces growth hormone and adrenocorticotropic hormone (ACTH) release through actions in the hypothalamus.[305,306]

The 5-HT_{1A} receptor also has been of interest for its possible role in the response to antidepressant drugs.[307]

Animal models of stress and antidepressant drug effects have shown that 5-HT_{1A} receptor activation produces effects similar to those of antidepressants.[308] The hippocampal neurogenesis produced by various types of antidepressants similarly is thought to be mediated by 5-HT_{1A} receptors.[309]

The selective serotonin reuptake inhibitors (SSRIs) are the most frequently prescribed drugs to treat depression. They block serotonin reuptake, but their therapeutic action often requires weeks of treatment. This delay is thought to be the result of presynaptic and postsynaptic adaptive mechanisms secondary to reuptake inhibition. In particular, blockade of the serotonin reuptake transporter leads to increased concentrations of serotonin in the vicinity of the midbrain raphe nuclei. This elevated serotonin activates 5-HT_{1A} autoreceptors, thereby decreasing the firing rate of those cells and ultimately decreasing serotonin release in terminal projection fields.[310] Such an effect functionally counteracts the desired serotonin increase produced by the SSRIs in the projection fields. Long-term treatment with SSRIs leads to desensitization of 5-HT_{1A} autoreceptors with a return to a more normal cell firing pattern. Consequently, preventing this acute negative feedback mechanism has been hypothesized as a mechanism to enhance the clinical effect of SSRIs, including shortening the onset to therapeutic efficacy.[311] This approach has been examined in several clinical trials by administering the nonselective 5-HT_{1A} antagonist pindolol along with an SSRI. Results have been somewhat encouraging but not definitive. A meta-analysis indicated that pindolol may shorten the onset of action of an SSRI in depression within the first two weeks of treatment, a length of time that correlates well with the delay in effect observed for SSRIs.[312]

The 5-HT_{1A} receptor also has been recently examined as a target for the treatment of schizophrenia. In postmortem schizophrenia patients, numerous studies have reported increases in 5-HT_{1A} receptor density in the prefrontal cortex, where these receptors are primarily expressed on pyramidal cells and produce hyperpolarization.[313] This up-regulation would likely signal that these receptors are not receiving adequate stimulation by serotonin. Thus, 5-HT_{1A} receptor agonists might address this apparent deficit.

There are two mechanisms whereby 5-HT_{1A} agonists might improve the treatment of schizophrenia. The first involves attenuation of catalepsy, that is, reduction of the parkinsonian symptoms induced by the dopamine D_2 receptor blockade produced by antipsychotics. This action is thought to be necessary for their therapeutic efficacy but can produce motor effects resembling Parkinson's disease. $5HT_{1A}$ agonists can attenuate catalepsy in animals[314] and inhibit haloperidol-induced catalepsy in rodents.[315] In addition to their antagonist activity at dopamine D_2 receptors, the newest atypical antipsychotic medications have potent agonist effects at 5-HT_{1A} receptors.[313,316,317] It has therefore been suggested that the reduced incidence of motor side effects in atypical antipsychotic drugs such as clozapine, quetiapine, and ziprasidone might be due to their inherent 5-HT_{1A} receptor agonist properties.[313]

In addition to improving the parkinsonian symptoms that accompany treatment with many antipsychotics, 5-HT_{1A} agonists also have been shown to increase dopamine release in the prefrontal cortex in rodents, an effect that might be expected to improve the negative symptoms of schizophrenia. The atypical antipsychotics clozapine, olanzapine, and ziprasidone (but not haloperidol) enhanced DA release in the prefrontal cortex of wild-type but not 5-HT_{1A} knockout mice after systemic and local (clozapine and olanzapine) administration in the mPFC.[318] A number of studies have now demonstrated the ability of 5-HT_{1A} agonists to increase extracellular dopamine in the prefrontal cortex of rodent models. Although no new antipsychotic agent has been introduced since this perspective has been developed, there are several efforts to design novel atypical antipsychotic agents that have a mixed D_2 antagonist−5-HT_{1A} agonist profile of action.[319,320]

Another area where 5-HT_{1A} receptors play an important role is in the effects of psychostimulants, including addiction.[321] Psychostimulants such as amphetamine and cocaine induce the neuronal release not only of catecholamines but also of serotonin, which can activate 5-HT_{1A} receptors. Activation of 5-HT_{1A} autoreceptors facilitates psychostimulant addiction-related behaviors by reducing serotonin tone in projection areas, whereas postsynaptic 5-HT_{1A} receptors have a direct action.

Finally, there has been recent interest in the potential of 5-HT_{1A} agonists to serve as neuroprotective agents to prevent ischemic damage in brain.[322–327] There is now evidence to suggest that activation of adenylyl cyclases following reperfusion after ischemic attacks may be a fundamental effect involved in the neurotoxicity process.[328] Activation of the 5-HT_{1A} receptor, because it leads to inhibition of cyclases, might be expected to attenuate the effects of excess adenylyl cyclase activation.

2.3.2. The 5-HT_{1B} Receptor

Based upon early ligand binding studies, the 5-HT_{1B} receptor was believed to be absent in humans but present in rodents.[329] Although predictions had been made that the 5-HT_{1B} receptor was a species homologue of the 5-HT_{1D} receptor,[330] it was not until the cloning and sequencing of both the rat 5-HT_{1B} receptor and the human 5-$HT_{1D\beta}$ receptor that it was discovered the 5-$HT_{1D\beta}$ receptor was in fact the ortholog to the rodent 5-HT_{1B} receptor.[331,332] Accordingly, the 5-$HT_{1D\beta}$ receptor was renamed the human 5-HT_{1B} receptor. Although the human and rat orthologs share 93% identity, a difference in only one residue, an asparagine instead of a threonine in the seventh transmembrane domain of the human and rat receptors, respectively, is responsible for distinct differences in receptor pharmacology.

In the vasculature, cerebrovascular receptors that mediate 5-HT-induced constriction in human cerebral arteries were found to be of the 5-HT_{1B} type. Immunocytochemistry of cerebral arteries showed dense 5-HT_{1B} receptor immunoreactivity within the smooth muscle wall of human cerebral arteries.[333] This localization proved problematic for the first generation triptan drugs for migraine, as drugs like sumatriptan had significant 5-HT_{1B} agonist activity, in addition to their efficacy against migraine mediated by the 5-HT_{1D} receptor (see later).

Within brain neurons, 5-HT_{1B} receptors are presynaptic and are localized on axon terminals.[334,335] Autoradiographic and immunological studies in rat brain showed the presence of this receptor at high levels in the basal ganglia, especially the globus pallidus and substantia nigra. Moderate levels of receptor were detected in superior colliculus, enteropeduncular nuclei, and periaqueductal gray. In the cerebral cortex, hypothalamus, amygdala, and dorsal horn of the spinal cord, only low levels of 5-HT_{1B} receptor were detected.[336] Additionally, mRNA for this receptor is expressed in the striatum, raphe nuclei, pyramidal layer of CA1

in the hippocampus, cingulate cortex, retinal ganglion cells, subthalamic nucleus, nucleus accumbens, olfactory tubercle, and Purkinje cells in the cerebellum.[336] In addition to serving as presynaptic heteroreceptors on nonserotonergic neurons, for example, GABA and glutamatergic neurons, 5-HT_{1B} receptors function as autoreceptors to modulate serotonin release from axon terminals of serotonergic neurons in the raphe nuclei.[336,337]

Early pharmacological studies screening for antiaggressive drugs, combined with receptor pharmacology, indicated that the 5-HT_{1B} receptor was a key regulator of aggression.[338] Although activation of postsynaptic 5-HT_{1B} receptors decreases aggressive behavior, specific antagonists, surprisingly, have no effect.[337] This finding suggests that under normal baseline conditions, there is little serotonergic tone at 5-HT_{1B} receptors. 5-HT_{1B} receptor knockout mice display a hyperaggressive phenotype.[339,340] The increase in observed aggressive behaviors in the 5-HT_{1B} knockout mouse may actually be due, however, to an increase in impulsive behaviors and defects in impulsivity regulation rather than aggression per se.[341,342]

The 5-HT_{1B} receptor also is involved in learning and memory processes. Whereas activation of the receptor with specific agonists decreases performance, antagonists and the knockout mouse show enhanced performance in learning and memory tests, possibly through mechanisms involving modulation of cholinergic neurotransmission.[343–345]

With some parallels to the function of 5-HT_{1A} receptors, a role for 5-HT_{1B} receptors has been proposed in the action of SSRI-type antidepressant medications. In rats, chronic SSRI treatment down-regulates 5-HT_{1B} autoreceptor mRNA expression in raphe nuclei but not expression of heteroreceptor mRNA in other regions of the brain such as cortex or striatum.[346,347] Furthermore, administration of 5-HT_{1B} receptor antagonists augments the effects of SSRIs in rodent brain.[348,349] In knockout mice, SSRIs elicit an enhanced response in the tail suspension assay compared with wild-type; however, there is no change in response in the forced swim test.[350,351] These data, together, suggest that administration of a 5-HT_{1B} receptor antagonist with an SSRI in humans may enhance or decrease the time to onset of antidepressant effects. A further role for the 5-HT_{1B} receptor related to its modulation of the effects of SSRIs is in ejaculatory control, where antagonists of this receptor may have efficacy in treating premature ejaculation.[352–354]

There also is evidence for involvement of 5-HT_{1B} receptors in the response to drugs of abuse such as cocaine and ethanol. Agonists decrease alcohol consumption, whereas the knockout mouse shows increased consumption.[355,356] With cocaine, however, 5-HT_{1B} receptor agonists potentiate some of its effects, including self-administration.[357,358] Furthermore, whereas antagonists can decrease some of the effects of cocaine,[358] the 5-HT_{1B} knockout mouse shows an increased response, possibly due to compensatory changes in neurochemistry.[359] Potential mechanisms for these effects involve 5-HT_{1B} receptor modulation of dopamine and GABA function in the nucleus accumbens.[360]

2.3.3. The 5-HT_{1D} Receptor

The human 5-HT_{1D} (first named 5-$HT_{1D\alpha}$) receptor was first cloned by Hamblin and Metcalf in 1991.[361] Early radiographic visualization of 5HT_{1D} sites in human brain was reported by Waeber et al.[362] Using [^{3}H]5-HT and several masking ligands, these workers identified highest expression in the basal ganglia and substantia nigra. In rat and mouse brain, 5-HT_{1D} mRNA also was detected in the basal ganglia and in layer IV of the cortex, but the levels were relatively low in all brain areas examined.[363] 5-HT_{1D} receptor expression is fairly limited throughout the human brain[364] but can be detected on trigeminal fibers in the spinal trigeminal tract in the human brainstem.[365]

The 5-HT_{1D} receptor is of particular interest because it was identified as a target for the antimigraine drug sumatriptan, as well as the later so-called triptan drugs. Although it is still not clear whether migraine is primarily a vascular or a neurological dysfunction, it is known that the plasma levels of serotonin decrease during migraine, along with carotid vasodilatation. Conversely, an i.v. infusion of 5-HT can abort migraine.

The treatment of migraine was revolutionized by the introduction of sumatriptan and then second generation triptans such as zolmitriptan, rizatriptan, and naratriptan, all shown to be 5-$HT_{1B/1D/1F}$ receptor agonists. Bouchelet et al.[366] used PCR amplification to demonstrate the presence of sumatriptan-sensitive 5-HT receptors in postmortem human trigeminal ganglia and cerebral blood vessels. Their results showed the 5-HT_{1B} (originally 5-$HT_{1D\beta}$) receptor to be predominant in human cerebral blood vessels, with expression in both neural and vascular tissues. The 5-HT_{1D} receptor was found to be preferentially expressed in neural versus vascular tissues. Neural 5-HT_{1D} or 5-HT_{1F} receptors localized prejunctionally on trigeminovascular afferents appear to mediate the triptan-induced inhibition of the neurogenic inflammatory response, although additional sites of action may be involved in inhibiting the central transmission of pain for brain penetrant 5-HT_1 receptor agonists.[367] The antimigraine effect of the triptans is primarily thought to arise through cranial vasoconstriction and presynaptic inhibition of the trigeminovascular inflammatory responses implicated in migraine. Cerebral vasoconstriction through activation of 5-HT_{1B} receptors, however, cannot be discounted as a component of the mechanism of action of triptans, because all triptans have high affinity for this receptor. Unfortunately, the activity of sumatriptan and other triptans at 5-HT_{1B} receptors can also produce carotid vasoconstriction, a potentially dangerous side effect.[367] Selective 5-HT_{1D} and 5-HT_{1F} receptor agonists, lacking 5-HT_{1B} agonist activity, inhibit the trigeminovascular system without producing vasoconstriction. Thus, the most recent research for migraine therapies has focused on selective 5-HT_{1D} and 5-HT_{1F} receptor agonists that lack 5-HT_{1B} agonist activity. Nevertheless, one selective 5-HT_{1D} agonist (PNU-142633) proved ineffective in the acute treatment of migraine.[368]

2.3.4. The 5-HT_{1E} Receptor

The 5-HT_{1E} receptor was originally defined as a [^{3}H]5-HT binding site in human cortical tissue that was insensitive to 100 nM 5-carboxytryptamine (5-CT).[369,370] Cloning of the human 5-HT_{1E} receptor gene was reported later, in 1992.[371] Interestingly, the 5-HT_{1E} receptor is not expressed in some species. Although it has been shown to be expressed in human and guinea pig, it has not been found in rat or mouse.[372] Indeed, there is not even a gene for the 5-HT_{1E} receptor in the mouse genome![372]

The 5-HT_{1E} and 5-HT_{1B} receptors share approximately 60% identity in their transmembrane domains but have distinct pharmacological properties. Studies of chimeric 5-HT_{1E}/5-HT_{1B} receptors revealed that TM6 and TM7

contained sequence differences responsible for their differential affinity for 5-carboxamidotryptamine. Specifically, two amino acids in TM6, Lys310 and Glu311 in the 5-HT_{1E} receptor, corresponding to Ile333 and Ser334 in the 5-HT_{1B} receptor, are primarily responsible for the different affinities of 5-CT and several other 5-HT_{1E} and 5-HT_{1B} ligands.[373]

The distribution of 5-HT_{1E} binding sites was originally mapped out in human brain tissue using the radioligand [^{3}H]5-HT to label all 5-HT_1-type sites and masking with the 5-HT_{1A} ligand 8-OH-DPAT. Displacement of additional radioactivity by 5-CT represented 5-HT_{1D} sites, with the remaining specific binding designated as 5-HT_{1E} receptors.[296] 5-HT_{1E} sites were distributed in cortical layers II−VI (21−34% of specific [^{3}H]5-HT binding). In the CA1 and CA2 fields and dentate gyrus of the hippocampus, 5-HT_{1E} represented only a minor fraction of the specific [^{3}H]5-HT binding, but in the CA3−CA4 fields, 5-HT_{1E} sites represented a significant fraction (27%). The highest densities of 5-HT_{1E} sites were measured in subiculum and in entorhinal cortex, where 5-HT_{1E} sites represented the major binding in layer III. They also were present in layers II and IV (29% and 24%) and, to a lesser extent, in layers V and VI. No 5-HT_{1E} sites were detected in choroid plexus.[296] In situ hybridization histochemistry has been used to visualize 5-HT_{1E} receptors in cortical areas, caudate, putamen, and amygdala.[363]

Expression in these brain areas suggests that the 5-HT_{1E} receptor may play a role in cognition and memory processes. Unfortunately, we know almost nothing about the function of the 5-HT_{1E} receptor. A search of the National Library of Medicine (NLM) revealed that since the first proposal for the existence of the 5-HT_{1E} receptor in 1989, only 56 papers have been published that included the term 5-HT_{1E} in any search field. Sadly, one must again point to the lack of ligands that are specific for this receptor as a major explanation for our lack of knowledge about its role in normal brain physiology. Recent attempts to design 5-HT_{1E}-selective agonists have so far not been successful.[374] Futhermore, a knockout animal model has not yet been created for the 5-HT_{1E} receptor.

2.3.5. The 5-HT_{1F} Receptor

Adham et al.[375] first cloned the gene for the human 5-HT_{1F} receptor. This receptor has become important in recent years as a target for newer antimigraine drugs that may lack some of the side effects associated with earlier drugs such as sumatriptan. Early studies of the binding profile of the antimigraine drug sumatriptan found that it displayed highest affinity for 5-HT_{1D} and 5-HT_{1B} binding sites, indicating these to be the likely basis for its efficacy in the acute treatment of migraine.[376] Subsequent reports identified the 5-HT_{1D} receptor as the therapeutic target, with 5-HT_{1B} receptor agonism responsible for undesirable constriction of coronary arteries. Later studies, however, found that sumatriptan also has significant affinity for the human 5-HT_{1F} receptor.[377]

Autoradiographic studies with [^{3}H]sumatriptan in postmortem human brain sections revealed expression of this receptor in globus pallidus = substantia nigra > cortex > putamen > hippocampus.[378] In another autoradiographic study examining the brainstem, expression was seen in substantia nigra. The spinal cord, the spinal trigeminal nucleus, substantia gelatinosa, and the nucleus of the tractus solitarius and periaqueductal gray also showed significant levels of [^{3}H]sumatriptan binding.[364]

Using polymerase chain reaction (PCR) amplification, Bouchelet et al.[366] investigated the expression of sumatriptan-sensitive 5-HT receptors in postmortem human trigeminal ganglia and cerebral blood vessels. Although they reported that the 5-$HT_{1D\beta}$ (5-HT_{1B}) receptor is the dominant species in human cerebral blood vessels, it is expressed along with the 5-HT_{1F} receptor in both neural and vascular tissues. In the discussion of their results, these workers emphasized the importance of understanding better the role of the 5-HT_{1F} receptor in cerebrovascular functions and dural inflammation and pointed to its possible role in migraine.[366]

Increasing attention was then directed to the potential importance of the 5-HT_{1F} receptor as a target for migraine therapies. In experiments with a guinea pig model of migraine that examined 5-HT_{1B}, 5-HT_{1D}, and 5-HT_{1F} receptor subtypes, antagonist potencies at inhibiting neurogenic inflammation showed a significant positive correlation only with the 5-HT_{1F} receptor.[379] Further, in situ hybridization demonstrated 5-HT_{1F} receptor mRNA in guinea pig trigeminal ganglion neurons. The authors of this study interpreted their data to suggest the 5-HT_{1F} receptor as a rational target for migraine therapeutics.[379] From a series of compounds known to be effective against migraine, LY334370 was found to be the most potent in a [^{35}S]GTPγS binding assay developed for the cloned human 5-HT_{1F} receptor.[380] Wainscott et al.[381] later reported the development of [^{3}H]-LY334370 as a selective high-affinity radioligand for labeling 5-HT_{1F} receptors. Side-by-side comparison of [^{3}H]LY334370 and [^{3}H]sumatriptan showed labeling in the same brain regions. Preliminary studies in rhesus monkey and human brain showed [^{3}H]LY334370 binding in cortical layers IV−V, subiculum (in the monkey), and the granule cell layer of the cerebellum.[382]

High interest remains in the development of selective 5-HT_{1F} agonists that may relieve acute migraine without coronary vasoconstriction. Ramadan et al.[383] report a high correlation between the potency of 5-HT_1 receptor agonists in the guinea pig dural plasma protein extravasation assay and their 5-HT_{1F} receptor affinity. Further, they point out that 5-HT_{1F} receptors are expressed in the trigeminal system and may participate in blocking migraine pain transmission through the trigeminal ganglion and nucleus caudalis. They further suggest that activation of 5-HT_{1F} receptors on glutamate-containing neurons might inhibit glutamate release, which also could play a role in migraine. Finally, they note that selective 5-HT_{1F} receptor agonists such as LY334370 and LY344864 are effective in preclinical migraine models, LY334370 is effective clinically, and these drugs do not cause coronary vasoconstriction.[383]

2.3.6. The 5-HT_{5A} (and 5-HT_{5B}) Receptors

The human 5-HT_{5A} receptor was cloned in 1994 by Rees et al.[384] Although two 5-HT_5 receptors have been cloned, only the 5-HT_{5A} isoform has been identified in humans (as well as in rat and in mouse). A functional 5-HT_{5B} isoform is expressed in rat and mouse, but premature stop codons in the gene prevent a functional receptor from being expressed in humans. Francken et al.[385] and Hurley et al.[386] reported that the 5-HT_{5A} receptor expressed in HEK293 cells appeared to couple primarily through $G_{i/o}$ proteins to inhibit production of cAMP.

Expression of the 5-HT_{5A} receptor is essentially limited to the central nervous system (CNS), although it also has been found on neurons and neuronal-like cells of the carotid

body. In the CNS, the 5-HT_{5A} receptor is expressed rather broadly, with high densities in the olfactory bulb, neocortex, and medial habenula.[387] Pasqualetti et al.[388] used in situ hybridization histochemistry to study the regional distribution and cellular localization of 5-HT_{5A} receptor mRNA in human brain sections from autopsy samples. They observed that the main areas of 5-HT_{5A} mRNA expression were the cerebral cortex, hippocampus, and cerebellum. In the neocortex, 5-HT_{5A} receptor mRNA was primarily distributed in layers II, III, V, and VI. The dentate gyrus and the pyramidal cell layer of the CA1 and CA3 hippocampal fields also expressed high levels of 5-HT_{5A} mRNA. Broad distribution in these areas is consistent with the view that the 5-HT_{5A} receptor is involved in higher cortical and limbic functions. Wide distribution of 5-HT_{5A} mRNA also was detected in the cerebellum, with high expression in Purkinje cells, in the dentate nucleus, and, at lower density, in the granule cells. The authors noted that diffuse innervation of the cerebellum by serotonergic afferents might indicate that the 5-HT_{5A} receptor plays an important role in mediating the effects of 5-HT on cerebellar functions.[388] In rats, mRNA for the receptor was seen at high levels in the suprachiasmatic nucleus,[389] an area known to function as a circadian pacemaker in mammals. A 5-HT_{5A} receptor knockout mouse showed increased exploration of a novel environment but had a reduced locomotor response to the hallucinogenic drug LSD.[387]

The 5-HT_5 receptors have received perhaps the least study of all the serotonin receptor subtypes, and not a great deal is understood about their function.[390] Pharmacological study of these receptors again has been hampered primarily due to the lack of selective agonist ligands.[391] These receptors have highest affinity for the hallucinogen LSD, and 5-carboxamidotryptamine (5-CT) is more potent and has higher affinity than 5-HT itself.[390] These agonist ligands are very nonspecific, however, and bind to a wide range of other serotonin and, in the case of LSD, monoamine receptors.

Brain localization and functional studies potentially implicate this receptor in the control of circadian rhythms, mood, and cognitive function. It has been suggested that 5-HT_5 ligands might find utility in treating sleep disturbances or perhaps in attenuating the cognitive dysfunction in schizophrenia.[392] It has recently been reported[393] that certain 5-HT_{5A} antagonists produce behavioral effects in rodent models suggestive of potential antipsychotic activity, although the antagonists had no effect in classical dopaminergic models such as conditioned avoidance response disruption or blockade of apomorphine climbing or amphetamine-induced increases in motor activity. Nevertheless, further understanding of the role of this receptor in brain function must await additional research that will require the use of agonist and antagonist ligands specific for this receptor.

3. The 5-HT_3 Receptor, A Ligand-Gated Ion Channel

As noted earlier, it was recognized that in addition to the 5-HT_1 and 5-HT_2 families of receptors, there was a third category that was similar to "M"-type serotonin receptors originally named by Gaddum and Picarelli in 1957.[110] In contrast to all of the other serotonin receptors, which are GPCRs, the 5-HT_3 receptor is a cation-selective ion channel of the cysteine-loop transmitter-gated superfamily of ligand-gated ion channels that includes the acetylcholine nitotinic receptor, the anion-selective $GABA_A$ receptor, and glycine receptor.[394–397]

The 5-HT_3 receptor has significant homology to the nicotinic type of acetylcholine receptors, which also are members of the ligand-gated ion channel family (Figure 14). Because of the relatively high degree of homology, the physical structure of the 5-HT_3 receptor is thought to be very similar to that of the nicotinic acetylcholine receptor, for which a 4 Å cryo-electron microscopic image has been published.[398] Although the proposed topological organization of 5-HT_3 receptor subunits is based largely on hydropathy analysis and the homology to nicotinic acetylcholine receptors, studies with antibodies directed toward the loops connecting the helices in the subunits has provided direct evidence for an extracellular N-terminal domain and an intracellular loop between the third and fourth transmembrane domains, consistent with the structures of other ligand-gated ion channel subunit topological models.[399]

These receptors, located in both the peripheral and central nervous systems and found both pre- and postsynaptically, are comprised of five pseudosymmetrical subunits that surround a central ion channel (Figure 15). Each subunit is comprised of an extracellular site, which contains the ligand binding domain, a transmembrane region made up of four α-helices (M1−M4), and a cytoplasmic domain.[395,400,401] The binding site forms at the interface of two adjacent subunits, where three loops from one subunit and three β-strands from an adjacent subunit converge.[394]

The subunits can be either the same (homopentameric 5-HT_{3A} receptors) or different (heteropentameric 5-HT_{3A} or 5-HT_{3B} receptors). The 5-HT_{3A} subunits form functional homomeric receptors,[402] whereas the 5-HT_{3B} subunits are not functional without coexpression of 5-HT_{3A} subunits.[403] Recent evidence suggests that only the 5-HT_{3A} receptor subunit, and not the 5-HT_{3B} receptor subunit, is functionally present in the CNS.[404]

Homomeric 5-HT_{3A} receptors conduct mono- and divalent cations nonselectively and exclude anions.[405–407] Activation by serotonin leads to opening of the ion pore and a rapidly activating and then desensitizing inward current.[400,408]

Highest expression of 5-HT_3 receptors in the CNS is found in the spinal trigeminal nerve nucleus, area postrema (chemoreceptor trigger zone; CTZ), and solitary tract nucleus,[409,410] areas known to be critical for emesis. Early studies demonstrated 5-HT_3 binding sites in the human dorsal vagal complex but not in other medullar regions.[411] In rat brain, Gehlert et al.[412] found highest expression of the 5-HT_3 receptor in the brainstem, principally in the nucleus of the solitary tract. Slightly lower levels were observed in the area postrema, substantia gelatinosa of the trigeminal nucleus, and dorsal motor nucleus of the vagus.

The receptor also has been detected at lower levels in several areas of the forebrain, including the hippocampus, nucleus accumbens, putamen, caudate nucleus, and amygdala.[409,413–415] Within the hippocampus, highest levels were seen in the granule cell layer of the dentate gyrus.[409]

Although the 5-HT_3 receptor may be a potential therapeutic target for several disease indications, only two applications have thus far been developed: the treatment of nausea and emesis and of irritable-bowel syndrome (IBS). These therapeutics employ 5-HT_3 receptor antagonists,[416] and agonists are not likely to be developed because they induce nausea and produce anxiety. Since they were introduced in the early 1990s, 5-HT_3 antagonists such as ondansetron (Zofran) have

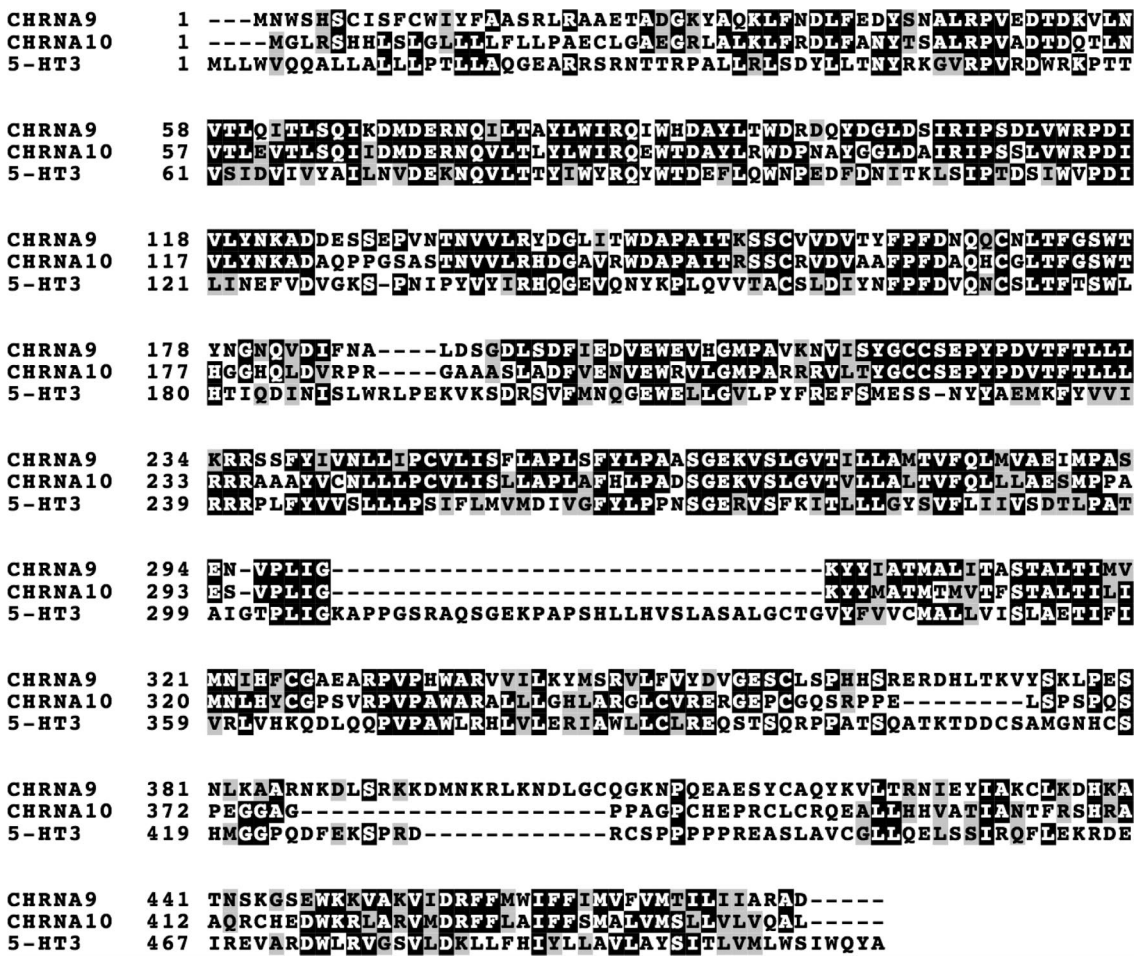


Figure 14. Alignment of the serotonin 5-HT_3 receptor sequence with the nicotinic acetylcholine receptor 9 and 10 α subunits.

become widely used for treating chemotherapy-induced emesis. Chemotherapeutic agents induce the peripheral release of large amounts of serotonin from enterochromaffin cells, which stimulates 5-HT_3 receptors on vagal afferents, in the solitary tract nucleus (STN), and in the chemo-receptor trigger zone (CTZ). These areas then send signals to the emetic center in the medulla.

Within the gut, 5-HT_3 receptors mediate fast neurotransmission within the enteric nervous system, as well as stimulate mucosal processes of myenteric primary afferent neurons.[417] Antagonists of 5-HT_3 receptors, such as alosetron, are therefore constipating and have been used clinically in the treatment of IBS with diarrhea.[418,419] For treatment of IBS with constipation, 5-HT_4 receptor agonists, as discussed earlier, can be effective.[246]

Expression of the 5-HT_3 receptor in forebrain areas indicates that this receptor may play a role in higher cognitive processes, and receptor antagonists might be useful in treating schizophrenia and psychostimulant abuse.[420] Although 5-HT_3 antagonists seem to have no effect in normal animals, they do have actions in animals with abnormal behavior, particularly where dopamine function has been increased.[421–423] For example, direct activation of the rat mesolimbic dopamine system by injection of a substance P analogue directly into the ventral tegmental area produced hyperlocomotion that was antagonized by a 5-HT_3 receptor antagonist.[424]

In vivo microdialysis studies have shown that direct microinfusion of serotonin into the rat nucleus accumbens leads to increased extracellular levels of dopamine in the accumbens.[425] The serotonin-induced increase in dialysate DA was attenuated by coperfusion with a specific 5-HT_3 receptor antagonist. Similarly, direct perfusion of a 5-HT_3 agonist into the nucleus accumbens enhanced extracellular dopamine release, and coperfusion of a 5-HT_3 antagonist blocked this effect. When the serotonin terminals were lesioned, the effect still occurred, suggesting location of 5-HT_3 receptors on presynaptic dopamine terminals.[426]

Intraperitoneal administration of ethanol to rats increases extracellular dopamine concentrations in the nucleus accumbens. Local perfusion of ethanol directly into the nucleus accumbens also leads to increased extracellular dopamine. Adding a 5-HT_3 antagonist to the perfusate markedly attenuated the ethanol-stimulated release of DA.[427] These data suggest that the ability of ethanol to stimulate the release of DA in the nucleus accumbens, a key part of the mesocortical dopamine pathway, may be at least partially mediated by 5-HT_3 receptors. Thus, numerous studies provide evidence that 5-HT_3 receptor antagonists can reduce elevated mesolimbic dopamine activity by blocking 5-HT_3 receptors in terminal fields of the mesolimbic dopamine system.[428]

A number of studies in animal models also have demonstrated that 5-HT_3 receptor antagonists have an anxiolytic effect.[429–432] A clinical trial showed that 5-HT_3 receptor antagonists have anxiolytic effects in humans.[433] It also might be noted that 5-HT_3 receptor antagonists are effective in preventing the behavioral syndrome that follows withdrawal from treatment with diazepam, nicotine, cocaine, or alcohol.[422]

Although several antagonists are available for clinical use, at least in Europe, they are principally used for treating

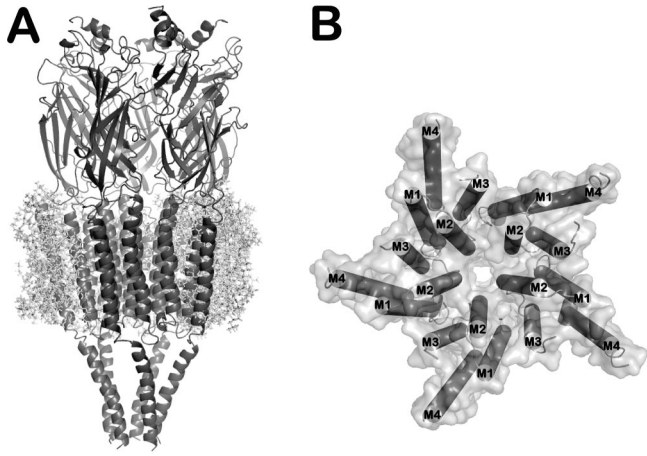


Figure 15. Representation of the serotonin 5-HT_3 receptor, showing its overall pentameric structure, where each subunit is comprised of four membrane-spanning helices. No crystal structure exists for this receptor, and the illustration is based on the 4 Å resolution cryoelectron microscopy image of the nicotinic acetylcholine receptor (pdb 2bg9).[398] Sequence homology and extensive other work indicates that the nicotinic acetylcholine receptor has high structural similarity to the 5-HT_3 receptor.[394] (A) Side view of a homology model of the 5-HT_3 receptor with a simulated membrane placed at the level of the transmembrane regions that comprise the central ion pore. The 5-HT binding protein is located extracellularly, on top of the transmembrane region, toward the top of the figure. The agonist binding site is located at the convergence of portions of the extracellular loops from two adjacent subunits. (B) This view looks down on the transmembrane pore region, with the 5-HT binding protein portion of the receptor removed. The helices are shown as cylinders, with a transparent van der Waals surface superimposed to indicate the size of the protein. Helix 2 (M2) in each subunit lines the ion conducting pore, which is clearly visible in the center of the bundle.

chemotherapy-induced nausea and vomiting and for IBS. Although there have been a few very limited clinical studies of the potential of 5-HT_3 receptor antagonists for the treatment of bulimia and pruritis, for improving memory and cognitive function, and for pain relief in fibromyalgia,[394] it is somewhat surprising that applications in treating these or various psychiatric disorders have not been further developed. Clearly, there is much more potential for therapeutic development based around 5-HT_3 receptor function in the CNS.

4. Conclusions and Perspectives

G-protein coupled receptors are arguably one of the most important classes of therapeutic targets; approximately 50% of currently marketed drugs act at GPCRs.[434] For CNS disorders, where drugs must have appropriate pharmacokinetic properties and be brain penetrant, it is likely that small molecule agonists and antagonists for GPCRs will continue to be a mainstay of the pharmaceutical industry.

The phylogenetically old serotonin systems have been conserved through evolution because their functions serve as the cornerstone of many fundamental mechanisms related to survival of the species: feeding, reproduction, and homeostasis. As evolution progressed, serotonin systems acquired expanded roles in regulation of mood states and cognition. There seems little doubt, in view of the important roles that have already been identified for serotonin neurotransmission in both the CNS and periphery, that future research will uncover additional essential functions of particular serotonin-driven signaling systems. This conclusion seems inescapable, particularly when one realizes that we still lack suitable agonist and antagonist ligands that are specific for only one subtype of most of the serotonin receptors. Further understanding of the structure and function of G-protein coupled receptors, and application to serotonin receptors, ultimately should lead to structure-based design principles that allow de novo design of badly needed ligands and, from there, to novel therapies. The ready availability of increasingly powerful computers and molecular modeling software makes this an exciting time for medicinal chemists and pharmacologists alike!

In addition, with the techniques available in the postgenomic era, researchers are rapidly developing an understanding of genetic regulatory mechanisms, and how serotonin receptors vary between individuals both in expression and function, and how a single change in receptor structure might modulate phenotypes. These types of studies may some day lead to novel therapeutics tailored to treat more effectively certain classes of responders based upon their specific receptor amino acid sequence. The continuing development of new genetic model systems in mammalian as well as invertebrate systems also will dramatically enhance the rate of understanding of the molecular mechanisms underlying serotonergic function.

The field of serotonin research had its beginnings in the study of a component of intestinal tissue that caused smooth muscle contraction. From that point, progress in science has led to discovery of many of the functions of serotonin in the CNS, including roles in memory, cognition, mood regulation, and likely involvement in psychiatric disorders. We can only be awed by the diversity of function accorded to this simple molecule by the forces of evolution. But, despite the tremendous technical advances of the past several decades, it must be admitted that we are still in a sort of "dark ages" with respect to understanding the brain and the neurochemical and physiological basis of cognition, and no doubt the next 60 years of serotonin research will be even more exciting!

5. Acknowledgments

The authors would like to acknowledge support for their serotonin-related research provided by NIH Grants DA02189 (D.E.N) and MH078454 (C.D.N.).

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CR078224O