

# SEROTONIN

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Serotonin, or 5-hydroxytryptamine (5-HT), has been implicated in almost every conceivable physiologic or behavioral function—affect, aggression, appetite, cognition, emesis, endocrine function, gastrointestinal function, motor function, neurotrophism, perception, sensory function, sex, sleep, and vascular function (1). Moreover, most drugs that are currently used for the treatment of psychiatric disorders (e.g., depression, mania, schizophrenia, autism, obsessive-compulsive disorder, anxiety disorders) are thought to act, at least partially, through serotonergic mechanisms (see elsewhere, *this volume*). How is it possible for 5-HT to be involved in so many different processes? One answer lies in the anatomy of the serotonergic system, in which 5-HT cell bodies clustered in the brainstem raphe nuclei are positioned through their vast projections to influence all regions of the neuraxis. Another answer lies in the molecular diversity and differential cellular distribution of the many 5-HT receptor subtypes that are expressed in brain and other tissues.

During the past decade, molecular cloning techniques have confirmed that putative 5-HT receptor subtypes, predicted from radioligand binding and functional studies (e.g., 5-HT<sub>1</sub>, 5-HT<sub>2</sub>, 5-HT<sub>3</sub>, 5-HT<sub>4</sub>), represent separate and distinct gene products. This knowledge has revolutionized contemporary research on the serotonergic system. Through the use of *in situ* messenger RNA (mRNA) hybridization and immunocytochemical maps, studies of previously recognized 5-HT receptors could be directed more precisely toward neurons and model cell lines that express these specific 5-HT receptor subtypes. Moreover, by the use of cloning techniques, investigations could be initiated to determine the functional role of previously unrecognized 5-HT receptors (e.g., 5-HT<sub>5</sub>, 5-HT<sub>6</sub>, 5-HT<sub>7</sub>). Concurrently, much progress has been made in delineating the signal transduction pathways of the various 5-HT-receptor subtypes. The focus of this review is on the molecular and

cellular aspects of individual 5-HT receptor subtypes and their transduction mechanism, in addition to interactions between different receptor subtypes within a single neuron or region. The implications of this work in understanding the global functions of the 5-HT system are discussed.

## 5-HT RECEPTOR SUBTYPES: MOLECULAR AND CELLULAR ASPECTS

### Molecular Biology

In the first half of the last decade, the cloning of the major known families of 5-HT receptors was accomplished. More recently, attention has turned to issues of transcriptional and post-transcriptional regulation.

### RNA Processing

The 5'-flanking region of several 5-HT-receptor genes has been cloned, and consensus sequences for transcription factors have been identified in the promoter region (2–4). The identification of these potential regulatory sites sets the stage for investigations on possible functionally significant regulation of gene transcription *in vivo* (5). A prominent form of post-transcriptional regulation is alternative RNA splicing, in which the splicing out of intronic sequence varies. Alternative splicing is common and occurs for a number of 5-HT receptors, including the 5-HT<sub>2C</sub>, 5-HT<sub>4</sub>, and 5-HT<sub>7</sub> receptors. The two splice variants of the 5-HT<sub>2C</sub> receptor described in the literature encode severely truncated proteins with no obvious function (6–8). In contrast, the splice variants of the 5-HT<sub>4</sub> receptor (5-HT<sub>4(a)</sub>–5-HT<sub>4(f)</sub>) and 5-HT<sub>7</sub> receptor (5-HT<sub>7(a)</sub>–5-HT<sub>7(d)</sub>) differ in length and composition in the carboxyl terminus (see refs. 9 and 10 for review). Marked species differences and perhaps regional differences lead to different patterns of splicing. Recently, Claeysen et al. (11) showed that the shortest 5-HT<sub>4</sub> receptor variants have the highest degree of constitutive activity, suggesting that the long tail provides structural stability to the molecule. Splice variants of the 5-HT<sub>7</sub> receptor have no known functional differences. In contrast, a second form of post-transcriptional regulation, RNA editing, tends to

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have marked effects on the functional properties of proteins. For example, RNA editing changes a single amino acid in the  $\beta$  subunit of the AMPA ( $\alpha$ -amino-3-hydroxy-5-methyl-4-isoxazole propionic acid) receptor, which dictates the gating properties of this ligand-gated ion channel (see ref. 12 for review).

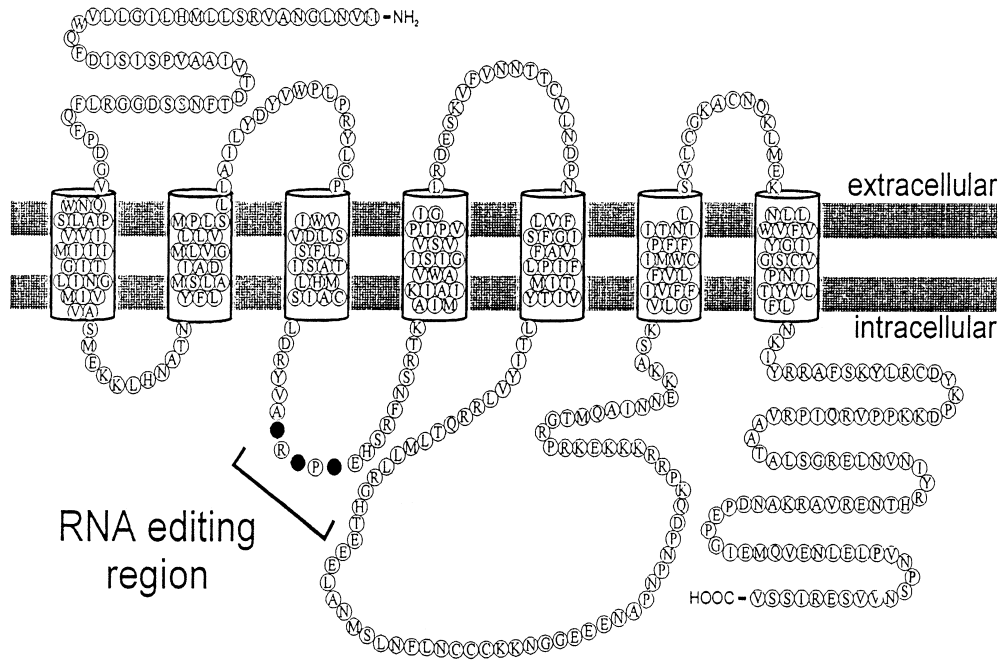
RNA editing in mammalian systems was discovered about a decade ago and is defined as any modification, other than alternative splicing, that occurs at the level of mRNA. Several mechanisms of RNA editing exist, but mammalian editing generally involves the conversion of adenosine residues to inosines by the action of a family of adenosine deaminases (13). Such editing events have the potential to alter the genetic code at the level of RNA; the resulting is the formation of multiple protein isoforms with altered function. The discovery of RNA editing of the 5-HT<sub>2C</sub> receptor provided the first, and so far only, example of editing of a G protein-coupled receptor (14). Editing of the human 5-HT<sub>2C</sub> receptor mRNA involves five sites, *A* through *E*, where adenosine is converted to inosine; inosine substitutes for guanosine in the genetic code, thus generating different protein isoforms. Multiple RNA isoforms have been found for the 5-HT<sub>2C</sub> receptor in human brain, predicting the formation of protein isoforms with up to three amino acids changed in the second intracellular loop of the receptor (15, 16). Editing at the *A*, *B*, *C*, and *D* adenosine residues of human 5-HT<sub>2C</sub>-receptor mRNA leads to predicted changes in all three amino acids to yield valine, serine, valine (VSV) at positions 156, 158, and 160 rather than isoleucine, asparagine, isoleucine (INI) at these positions in the unedited receptor isoform (Fig. 2.1). Editing at all five sites predicts the formation of the valine, glycine, valine (VGV) isoform. Because the second intracellular loop has been implicated in receptor–G protein coupling, initial functional studies have focused on the intracellular signaling properties of 5-HT<sub>2C</sub>-receptor isoforms. These studies have shown that edited receptor isoforms couple less efficiently to G<sub>q</sub> proteins, evidenced by lowered agonist potencies to activate phospholipase C (7,14,15) and reduced receptor constitutive activity (16,17). The discovery that the 5-HT<sub>2C</sub> receptor is regulated by RNA editing presents a challenge for pharmacologists because multiple isoforms with potentially different pharmacologic properties and functions are predicted to exist in brain. It is not clear, for example, which receptor isoform should be used for *in vitro* modeling of the receptor and to characterize newly developed drugs. The unedited INI isoform is predicted to represent less than 10% of the total population of receptors in human brain; the principal isoform is VSV (15,16). To date, all studies of function have involved recombinant cells expressing a single receptor isoform. Evaluation of the *in vivo* functional consequences of RNA editing of the 5-HT<sub>2C</sub> receptor awaits the development of experimental methods for isolating the function of a single, specific isoform in brain. Strategies such as the generation of blocking antibodies that target specific amino

acid combinations in the second intracellular loop or the development of transgenic mice that express a single isoform may be successful, although they are experimentally challenging and time-consuming. It is not known whether other 5-HT receptors, or for that matter other G protein-coupled receptors, are subject to RNA editing. It seems likely that the 5-HT<sub>2C</sub> receptor would not be unique. However, screening methods for reliably detecting RNA editing are not available; instead, the discovery of edited substrates depends on comparing genomic and cyclic DNA sequences. Consequently, new edited substrates are slow to emerge.



### *Post-translational Regulation*

Receptor desensitization and down-regulation are common adaptive responses to sustained agonist exposure. The most widely accepted model of desensitization of G protein-coupled receptors is based on extensive studies of the  $\beta$ -adrenergic receptor, a G<sub>s</sub>-linked receptor. In a simplified rendition of the model, agonist binding to a cell surface receptor leads to receptor phosphorylation, arrestin binding, receptor internalization into endosomes, dephosphorylation of the receptor, and recycling back to the cell surface. Receptor phosphorylation is thought to mediate desensitization by uncoupling the receptor from G protein. For many receptors, this phosphorylation event is promoted by a family of G protein-coupled receptor kinases (GRKs). However, second messenger-dependent kinases and protein kinases C and A, in addition to GRKs, have all been implicated in the desensitization of 5-HT<sub>1A</sub> receptor (18). Abundant *in vivo* studies have documented a blunting of 5-HT<sub>1A</sub> receptor-mediated behavioral responses after long-term treatment with agonists or serotonin uptake inhibitors that indirectly promote receptor activation. Indeed, desensitization of raphe 5-HT<sub>1A</sub> autoreceptors has been proposed to play a role in the delayed therapeutic onset of antidepressant drugs (see ref. 19 for review).

Protein kinase C has also been implicated in 5-HT<sub>2A</sub>-receptor desensitization (20). Subsequent steps in the desensitization–resensitization cycle have been demonstrated for the 5-HT<sub>2A</sub> receptor, including arrestin binding to the third intracellular loop of the receptor (21) and internalization into endocytotic vesicles (22). Surprisingly, 5-HT<sub>2A</sub>-receptor antagonists also cause receptor internalization, which may be related to the earlier findings of antagonist down-regulation of 5-HT<sub>2A</sub> receptors (see ref. 23 for review). Importantly, antagonist-mediated 5-HT<sub>2A</sub>-receptor internalization has been confirmed in cortical pyramidal cells and is accompanied by an apparent redistribution from dendrites to cell bodies (24). The fact that atypical antipsychotic drugs such as clozapine and olanzapine, but not haloperidol, promote 5-HT<sub>2A</sub>-receptor internalization has led to speculation that this novel antagonist property may be related to therapeutic action in schizophrenia.



Editing Sites		A	B		EC		D		
Genomic	GTA	GCA	ATA	CGT	AAT	CCT	AAT	GAG	CAT
mRNA	GUA	GCA	IUI	CGU	IIU	CCU	IUU	GAG	CAU
Non-Edited			I		N		I		
<u>Edited at Sites :</u>									
<u>Encoding Amino Acid</u>									
A or AB			V						
B			M						
C					S				
E					D				
CE					G				
D							V		

-  RNA editing sites  
 Site E editing only seen in Human isoform

**FIGURE 2.1.** RNA editing of the 5-hydroxytryptamine subtype 2C (5-HT<sub>2C</sub>) receptor. Editing of the 5-HT<sub>2C</sub>-receptor messenger RNA transcript generates multiple receptor isoforms that differ in one to three amino acids in the second intracellular loop.

## Receptor Constitutive Activity and Inverse Agonism

In *Psychopharmacology: The Fourth Generation of Progress*, the concept of inverse agonist properties of serotonin-receptor antagonists was novel and unique to the 5-HT<sub>2C</sub> receptor (25). *Inverse agonism* is the ability of certain antagonists to block the spontaneous (also referred to as *constitutive*) activity of a G protein-coupled receptor, in addition to blocking the binding of an agonist. These antagonists are referred to as *inverse agonists* because their effects are opposite to those of agonists. In contrast, other antagonists, referred to as *neutral antagonists*, have no apparent activity when added alone, even though they block the action of an agonist. To explain receptor constitutive activity and inverse agonism of antagonists, the model of receptor–G protein coupling was modified to propose spontaneous receptor isomerization to an active form ( $R^*$ ) in the absence of an agonist (26). Antagonists with inverse agonist activity bind to and stabilize the inactive receptor conformation ( $R$ ), whereas neutral antagonists were proposed to bind equally well to the active and inactive forms of the receptor. Since 1995, 5-HT<sub>1A</sub> receptors (27, 28), 5-HT<sub>1B</sub> receptors (29), 5-HT<sub>1D</sub> receptors (30), mutant 5-HT<sub>2A</sub> receptors (31), 5-HT<sub>4</sub> receptors (11,32), and 5-HT<sub>7</sub> receptors (33) have been shown to exhibit constitutive activity; both inverse agonists and neutral antagonists have been described for these receptors.

All these studies demonstrating inverse agonist properties of 5-HT antagonists have been performed *in vitro* in cells expressing recombinant receptors. It is not known whether inverse agonism is relevant to the *in vivo* actions of receptor antagonists, including their therapeutic properties. Recent *in vivo* studies of a simple motor reflex have produced convincing evidence for constitutive activity of the 5-HT<sub>2A/2C</sub> receptor and differential effects of inverse agonists versus neutral antagonists (34,35). However, Millan et al. (36) were unable to show differential effects of inverse agonists versus neutral antagonists at the 5-HT<sub>1B</sub> receptor and concluded that *in vitro* demonstrations of inverse agonist activity cannot be extrapolated to the *in vivo* situation. Recent studies by Berg et al. (37) suggest that even in the absence of measurable effects on basal activity, prolonged treatment with inverse agonists at the 5-HT<sub>2C</sub> receptor produces enhanced phospholipase C activation, likely because of increased expression of G<sub>q</sub> proteins.

## Electrophysiology

Although the electrophysiologic actions of 5-HT may seem quite varied, considerable uniformity is found within each of the major receptor families. For example, all members of the 5-HT<sub>1</sub> family tend to have inhibitory actions either presynaptically or postsynaptically. Similarly, all members of the 5-HT<sub>2</sub> family tend to have excitatory actions. There-

fore, the discussion of 5-HT electrophysiology is organized according to receptor family subtypes.

### 5-HT<sub>1</sub> Receptors

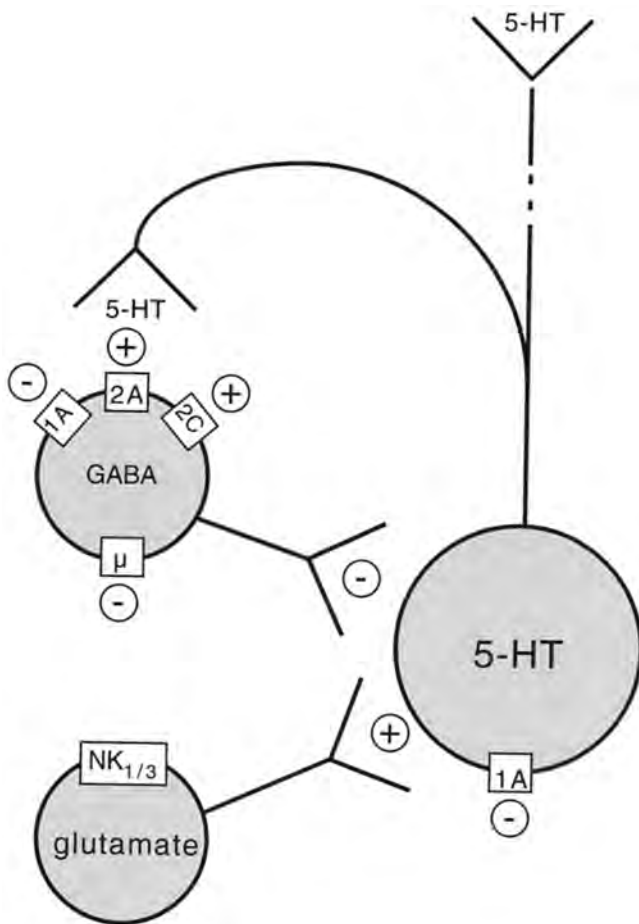
Dense concentrations of 5-HT<sub>1A</sub> binding sites and high levels of 5-HT<sub>1A</sub> mRNA expression are found in a number of regions, including the dorsal raphe nucleus, hippocampal pyramidal cell layer, and cerebral cortex (38–40). Studies in these regions have been useful in delineating the physiologic role of this receptor.

### Raphe Nuclei

Serotonergic neurons of the raphe nuclei are inhibited by the local (microiontophoretic) application of 5-HT to their cell body region. Thus, the receptor mediating this effect has been termed a *somatodendritic autoreceptor* (as opposed to the prejunctional autoreceptor). Early studies in the dorsal raphe nucleus showed that lysergic acid diethylamide (LSD) and other indolamine hallucinogens are powerful agonists at the somatodendritic 5-HT autoreceptor (41,42). Functionally, the somatodendritic 5-HT autoreceptor has been shown to mediate collateral inhibition (43). The ionic basis for the autoreceptor-mediated inhibition, either by 5-HT or LSD, is an opening of K<sup>+</sup> channels to produce a hyperpolarization (44); these channels are characterized by their inwardly rectifying properties (45). As in the dorsal raphe nucleus, serotonergic neurons of the lower brainstem are also hyperpolarized by 5-HT via the opening of inwardly rectifying K<sup>+</sup> channels (46,47). Similar findings in acutely isolated (48) and individually microcultured (49) dorsal raphe neurons underscore the fact that autoreceptor inhibition is independent of any inputs to the raphe nucleus. Patch-clamp recordings in the cell-attached and outside-out configuration from such acutely isolated dorsal raphe neurons show that the increase in K<sup>+</sup> current results from a greater probability of opening of unitary K<sup>+</sup> channel activity (50).

The somatodendritic autoreceptors of serotonergic neurons in both the dorsal raphe nucleus and the nucleus raphe magnus appear to be predominantly of the 5-HT<sub>1A</sub> subtype; a variety of drugs with 5-HT<sub>1A</sub> selectivity (e.g., 8-OH-DPAT and the anxiolytic drugs buspirone and ipsapirone) share the ability to inhibit raphe cell firing potently in a dose-dependent manner (47,50a,50b). Recently, a highly selective 5-HT<sub>1A</sub> antagonist (WAY 100635) has been found that potently blocks the direct inhibition of dorsal raphe serotonergic neurons both by 5-HT and selective 5-HT<sub>1A</sub> agonists (51,52). WAY 100635 also blocks the indirect inhibition of dorsal raphe neurons induced by selective 5-HT reuptake inhibitors (53). Complementing this autoinhibitory role of local 5-HT<sub>1A</sub> receptors is a long-loop negative-feedback system activated by postsynaptic 5-HT<sub>1A</sub> receptors in the medial prefrontal cortex (54,55).

In addition to long-loop feedback systems, short-loop regulatory circuits are found within the dorsal raphe nucleus and the adjacent periaqueductal gray (PAG). These short-loop circuits involve interactions between 5-HT and local inhibitory GABAergic ( $\gamma$ -aminobutyric acid) and excitatory glutamatergic neurons (56). Interestingly, both the local excitatory and inhibitory inputs to 5-HT cells are negatively modulated by  $\mu$  opiate receptors. Local GABAergic neurons are activated by 5-HT via 5-HT<sub>2A/2C</sub> receptors in a local, negative feedback loop that complements 5-HT<sub>1A</sub>-mediated autoinhibition (57). Neurokinins such as substance P and neurokinin B, via NK<sub>1</sub> and NK<sub>3</sub> receptors, respectively, activate mostly local glutamatergic excitatory inputs to 5-HT cells (58). Some of these local circuits are depicted schematically in Fig. 2.2.



**FIGURE 2.2.** Schematic representation of local regulatory circuitry within the dorsal raphe nucleus (DRN). In addition to somatodendritic 5-hydroxytryptamine subtype 1A (5-HT<sub>1A</sub>) autoreceptors on the 5-HT neurons *per se*, local GABAergic ( $\gamma$ -aminobutyric acid) and glutamatergic neurons in the DRN/ventral periaqueductal gray (PAG) region modulate the activity of serotonergic neurons. Note the location of inhibitory  $\mu$  opiate receptors on both categories of local neurons. Also depicted are excitatory 5-HT<sub>2A/2C</sub> and inhibitory 5-HT<sub>1A</sub> receptors on GABAergic neurons and excitatory NK<sub>1</sub> (substance P) and NK<sub>3</sub> (neurokinin B) receptors on glutamate neurons in the DRN/PAG.

### Other Subcortical Regions

Inhibitory or hyperpolarizing responses to 5-HT have been reported in a wide variety of neurons in the spinal cord, brainstem, and diencephalon. In general, such responses have been attributed to mediation by 5-HT<sub>1</sub> receptors. In sensory neurons of dorsal root ganglia, a 5-HT<sub>1</sub>-like receptor has been reported to reduce the calcium component of action potentials and to produce hyperpolarizations that can be mimicked by 5-HT<sub>1A</sub> agonists such as 8-OH-DPAT (59). In cerebellar Purkinje cells, 5-HT-induced inhibition, but not excitation, is mediated through 5-HT<sub>1A</sub> receptors (60). In brain slices of the nucleus prepositus hypoglossi, focal electric stimulation evokes inhibitory postsynaptic potentials (IPSPs) that are mediated by 5-HT<sub>1A</sub> receptors to activate an inwardly rectifying K<sup>+</sup> conductance (61) and a novel outwardly rectifying K<sup>+</sup> conductance (62). In the midbrain PAG, a region known to be involved in pain modulation and fear responses, approximately half the cells are inhibited/hyperpolarized by 8-OH-DPAT, suggesting mediation by 5-HT<sub>1A</sub> receptors (63). In the ventromedial hypothalamus (64) and lateral septum (65,66), 5-HT and 5-HT<sub>1A</sub> agonists produce inhibitory effects, also by activating a K<sup>+</sup> conductance. In addition to these postsynaptic effects, 5-HT has been shown to suppress glutamatergic synaptic transmission via presynaptic 5-HT<sub>1B</sub> receptors in various regions, including the hypoglossal nucleus (67) and the nucleus accumbens (68).

In the rat laterodorsal tegmental nucleus, bursting cholinergic neurons are hyperpolarized by 5-HT via 5-HT<sub>1</sub> receptors (69). In freely behaving rats, the direct injection of 5-HT into the laterodorsal tegmental nucleus has been found to suppress rapid-eye-movement (REM) sleep (70). In unanesthetized cats, a corresponding population of neurons that are active selectively during REM states (REM-on neurons) in the laterodorsal tegmental nucleus has been shown to be inhibited by direct application of the 5-HT<sub>1A</sub> agonist 8-OH-DPAT (71). It has been proposed that during REM sleep, the removal of a tonic inhibitory 5-HT influence from these cholinergic neurons may be responsible for the emergence of an activated EEG during this behavioral state.

### Hippocampus

Pyramidal cells of the CA1 region express high levels of 5-HT<sub>1A</sub>-receptor mRNA and 5-HT<sub>1A</sub>-receptor binding (72). Early on, intracellular recordings in brain slices showed that the 5-HT-induced inhibition was caused by hyperpolarization resulting from an opening of K<sup>+</sup> channels (73). Subsequent work, in which various pharmacologic approaches have been used in brain slices, has shown that the 5-HT-induced inhibition in both CA1 and CA3 pyramidal cells is mediated by the activation of receptors of the 5-HT<sub>1A</sub> subtype (74–77). After long-term but not short-term administration of various antidepressant treatments (selective 5-HT reuptake inhibitors, monoamine oxidase inhibitors,

tricyclic drugs, electroconvulsive therapy), disinhibitory responses are seen with the selective 5-HT<sub>1A</sub> antagonist WAY 100635, which suggests increased 5-HT<sub>1A</sub>-mediated inhibitory tone on CA3 hippocampal pyramidal cells (78). Interestingly, this increase in 5-HT<sub>1A</sub> tone after long-term antidepressant treatment is potentiated by short-term treatment with lithium (79).

In addition to the above-mentioned direct effects on pyramidal cells, 5-HT has been shown to depress both excitatory and inhibitory synaptic potentials in the hippocampus. Relatively high concentrations of 5-HT cause a reduction in electrically evoked excitatory postsynaptic potentials (EPSPs) in CA1 pyramidal cells (80), an effect that is mimicked by 8-OH-DPAT, which suggests mediation by 5-HT<sub>1A</sub> receptors. Indirect measures indicate that 5-HT acts presynaptically to reduce Ca<sup>2+</sup> entry and thereby glutamatergic synaptic transmission. In addition, a 5-HT<sub>1A</sub>-mediated inhibitory effect on putative inhibitory interneurons of the hippocampus has been observed (81,82). Consistent with an opening of K<sup>+</sup> channels, the inhibitory effects of 5-HT on interneurons result from a hyperpolarization associated with a reduction in input resistance. Functionally, the 5-HT<sub>1A</sub>-mediated inhibition of GABAergic interneurons in the hippocampus leads to a disinhibition of pyramidal cells in CA1. Clearly, the effects of 5-HT in the hippocampus are highly complex, involving both presynaptic and postsynaptic actions that may, to varying degrees, be inhibitory or disinhibitory, facilitative or disfacilitative.

### Cerebral Cortex

Hyperpolarizing/inhibitory responses in pyramidal cells of the cerebral cortex induced by 5-HT<sub>1A</sub> have been described in a number of studies. In entorhinal cortex, where the density of 5-HT<sub>1A</sub> receptors is especially high (and the density of 5-HT<sub>2A</sub> receptors low), unopposed 5-HT<sub>1A</sub> receptor-mediated hyperpolarizing responses are seen (83). However, cortical neurons in most other regions typically display mixed inhibitory and excitatory responses to 5-HT because of expression by the same pyramidal cells of multiple 5-HT receptor subtypes (e.g., 5-HT<sub>1A</sub> and 5-HT<sub>2/2C</sub>) (84–87). Hyperpolarizing responses mediated by 5-HT<sub>1A</sub> receptors are often unmasked or enhanced in the presence of 5-HT<sub>2</sub> antagonists, consistent with the idea that an interaction occurs between 5-HT<sub>1A</sub> and 5-HT<sub>2A</sub> receptors at an individual neuronal level (84,88,89). A similar suggestion of a shift in the balance between 5-HT-mediated excitation and inhibition comes from another *in vivo* study, in which both systemic and local application of 5-HT<sub>2</sub> antagonists was shown to prevent an enhancement of the unit activity (and cortical desynchronization) that normally occurs in response to noxious stimuli (tail compression) in anesthetized rats (90).

In addition to the above-mentioned postsynaptic effects, various presynaptic effects are mediated by 5-HT<sub>1</sub> receptors in the cerebral cortex. In cingulate cortex, 5-HT, acting

on presynaptic 5-HT<sub>1B</sub> receptors, reduces the amplitude of electrically evoked EPSPs, including both *N*-methyl-D-aspartate (NMDA) and non-NMDA components (87). Similar modulations of EPSPs, mediated by 5-HT<sub>1A</sub> or 5-HT<sub>1B</sub> receptors, have been reported for several cortical regions, including medial prefrontal (91) and entorhinal cortex (92).

### 5-HT<sub>2</sub> Receptors

Quantitative autoradiographic studies show high concentrations of 5-HT<sub>2</sub> binding sites and mRNA expression in certain regions of the forebrain, such as the neocortex (layers IV/V), piriform cortex, claustrum, and olfactory tubercle (93). With few notable exceptions (e.g., motor nuclei and the nucleus tractus solitarius), relatively low concentrations of 5-HT<sub>2</sub> receptors or mRNA expression are found in the brainstem and spinal cord. Studies aimed at examining the physiologic role of 5-HT<sub>2</sub> receptors in several of these regions are discussed in the following sections.

### Motoneurons

In the facial and other cranial motor nuclei, motoneurons have a high density of 5-HT<sub>2</sub>-receptor binding sites. Early studies *in vivo* showed that 5-HT applied microiontophoretically does not by itself induce firing in the normally quiescent facial motoneurons, but does facilitate the subthreshold and threshold excitatory effects of glutamate (94). Intracellular recordings from facial motoneurons *in vivo* or in brain slices *in vitro* (95,96) show that 5-HT induces a slow, subthreshold depolarization associated with an increase in input resistance, indicating a decrease in resting K<sup>+</sup> conductance. Ritanserin and other 5-HT<sub>2</sub> antagonists are able to block the excitatory effects of 5-HT in facial motoneurons selectively (97). Indolamine (e.g., LSD and psilocin) and phenethylamine (e.g., mescaline and DOI) hallucinogens act as 5-HT<sub>2</sub> agonists at facial motoneurons. Ionophoretically administered LSD, mescaline, and psilocin, although having relatively little effect by themselves, produce a prolonged facilitation of facial motoneuron excitability (98). Intracellular studies in brain slices show that the enhancement is in part caused by a small but persistent depolarizing effect of the hallucinogens (97,99).

### Other Subcortical Regions

In brain slices of the medial pontine reticular formation, 5-HT induces a hyperpolarization in some cells and a depolarization in other cells (100). The hyperpolarizing responses are associated with an increase in membrane conductance and have a 5-HT<sub>1</sub> pharmacologic profile. The depolarizing responses have a 5-HT<sub>2</sub> pharmacology and are associated with a decrease in membrane conductance resulting from a decrease in an outward K<sup>+</sup> current. These two actions of 5-HT do not appear to coexist in the same neurons because none of the cells display dual responses to selective agonists.

In brain slices of the substantia nigra pars reticulata, a majority of neurons are excited by 5-HT via 5-HT<sub>2</sub> receptors (101), possibly of the 5-HT<sub>2C</sub> rather than 5-HT<sub>2A</sub> subtype (102). Neurons in the inferior olivary nucleus are excited by 5-HT via 5-HT<sub>2A</sub> receptors, so that the oscillatory frequency of input to cerebellar Purkinje cells is altered (103). In the nucleus accumbens, the great majority of neurons are depolarized by 5-HT, and they are induced to fire (104). This depolarization is associated with an increase in input resistance secondary to a reduction in an inward rectifier K<sup>+</sup> conductance. Pharmacologic analysis shows that the depolarization is mediated by a 5-HT<sub>2-</sub> rather than a 5-HT<sub>1-</sub> or 5-HT<sub>3-</sub>-type receptor.

GABAergic neurons of the nucleus reticularis thalami show marked depolarizing responses to 5-HT, associated with a decrease in a resting or “leak” K<sup>+</sup> conductance; these excitatory responses are blocked by the 5-HT<sub>2</sub> antagonists ketanserin and ritanserin (105). The 5-HT-induced slow depolarization potently inhibits burst firing in these cells and promotes single-spike activity. It has been suggested that the 5-HT-induced switch in firing mode from rhythmic oscillation to single-spike activity, which occurs during states of arousal and attentiveness, contributes to the enhancement of information transfer through the thalamus during these states. GABAergic neurons within the medial septal nucleus are also excited by 5-HT via 5-HT<sub>2</sub> receptors (106). In the dentate gyrus of the hippocampus, a subpopulation of GABAergic neurons is activated via 5-HT<sub>2A</sub> receptors, evidenced by an increase in IPSP frequency in granule cells in the dentate gyrus (107). Recently, similar activation of GABAergic neurons via 5-HT<sub>2A</sub> receptors has been reported in the CA1 region of the hippocampus (108). These observations closely resemble findings in the piriform cortex, where a subpopulation of GABAergic interneurons is excited by 5-HT via 5-HT<sub>2A</sub> receptors (see below). Also, indirect evidence suggests that 5-HT-induced inhibition of dentate/interpositus neurons of the deep cerebellar nuclei is mediated indirectly by the activation of GABAergic interneurons through 5-HT<sub>2</sub> receptors (109). Taken together, these findings suggest that in multiple locations within the central nervous system, excitation of subpopulations of interneurons by 5-HT via 5-HT<sub>2</sub> receptors gives rise to indirect inhibitory effects.

### Cerebral Cortex

The electrophysiologic effects of 5-HT have been studied in several cortical regions. *In vitro* studies in brain slice preparations have shown that pyramidal cells in various cortical regions respond to 5-HT by either a small hyperpolarization, depolarization, or no change in potential (84–86, 110). Depending on the region of cortex under study, as described below, the depolarizations appear to be mediated by 5-HT<sub>2A</sub> or 5-HT<sub>2C</sub> receptors.

In addition to these postsynaptic effects, 5-HT induces an increase in “spontaneous” (not electrically evoked) post-

synaptic potentials or currents (PSPs/PSCs) in brain slices from various cortical regions. Originally, recordings were made from pyramidal cells in a paleocortical region, the piriform cortex. In that region, as in the hippocampus (see above), 5-HT, acting through 5-HT<sub>2A</sub> receptors, induces an increase in spontaneous IPSPs (86,111–115). *In vivo* studies have also provided evidence for a 5-HT<sub>2A</sub> receptor-mediated activation of GABAergic neurons in piriform cortex (116). As in piriform cortex, 5-HT can increase IPSCs in pyramidal cells in various layers of the neocortex (117, 118). The IPSCs result from the activation of cortical interneurons via 5-HT<sub>2A/2C</sub> or 5-HT<sub>3</sub> receptors (117). Immunocytochemical evidence has been found in primate cerebral cortex for a segregation of 5-HT<sub>2A-</sub> and 5-HT<sub>3-</sub>-expressing interneurons; the former project to somatobasilar and the latter to distal apical dendritic regions of pyramidal cells (119).

Quantitatively, in layer V pyramidal cells, synaptic events induced by 5-HT consist largely of EPSPs/EPSCs (118). Thus, approximately 85% of all PSCs are blocked by AMPA/kainate glutamate-receptor antagonists (e.g., LY293558) but not by the GABA<sub>A</sub> antagonist bicuculline (118). The 5-HT-induced increase in EPSCs is most pronounced in frontal regions, including the medial prefrontal cortex (118). In that region, 5-HT<sub>2A</sub> receptors are denser than in more posterior regions (40,120). Recent studies, in which intracellular labeling with biocytin was used, have confirmed that 5-HT-induced increases in EPSCs occur predominantly in layer V pyramidal cells, whereas responses are minimal in layer II/III cells and lacking in layer VI cells (121).

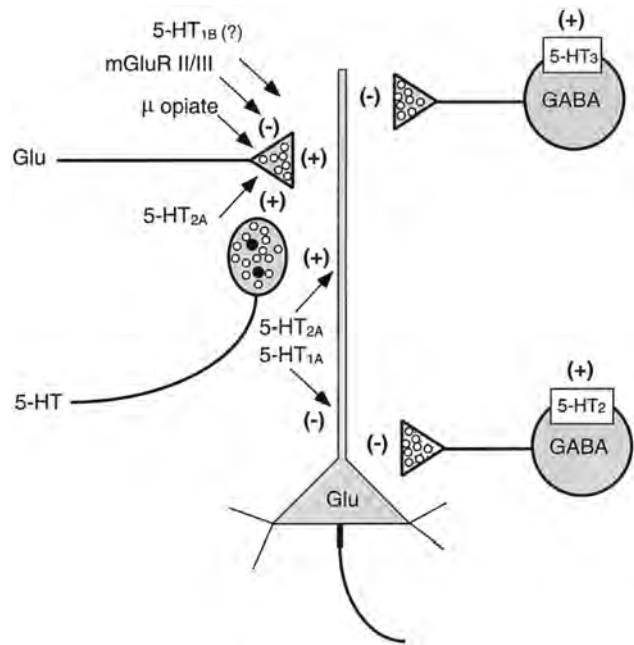
The 5-HT-induced EPSCs are antagonized competitively by low concentrations of the highly selective 5-HT<sub>2A</sub> antagonist MDL 100,907 (pA<sub>2</sub>, 8.8), which indicates mediation by 5-HT<sub>2A</sub> receptors (118,122). Norepinephrine, via α<sub>1</sub> adrenoceptors, also induces an increase in EPSPs in layer V pyramidal cells, but (at least in the rat) the increase is only a fraction of that produced by 5-HT (122). Changes in the frequency of synaptic currents or potentials are generally regarded as indicative of a modulation of presynaptic function. Accordingly, the nonspecific group II/III metabotropic glutamate receptor agonist (1S,3S)-ACPD (118) and the selective mGlu II/III metabotropic agonist LY354740 (123), which act at inhibitory autoreceptors and are located on glutamatergic nerve terminals, suppress the 5-HT-induced increase in the frequency of EPSCs. In addition, the activation of μ receptors, located presynaptically on thalamocortical inputs, also suppresses 5-HT-induced EPSCs, particularly in the medial prefrontal cortex (124). These results are consistent with the idea that activation of 5-HT<sub>2A</sub> receptors increases the release of glutamate onto layer V pyramidal cells through a presynaptic mechanism. 5-HT also produces a small but significant increase in the *amplitude* of spontaneous EPSCs, an effect that may involve postsynaptic amplification mechanisms (118). Such postsynap-

tic effects are consistent with the finding of a high level of 5-HT<sub>2A</sub>-receptor immunoreactivity in the apical dendrites of cortical pyramidal cells (125–127).

The 5-HT-induced EPSCs are blocked by bath application of the slice with the fast sodium channel blocker tetrodotoxin or perfusion with a solution containing no added calcium (“zero” calcium) (118). Ordinarily, tetrodotoxin sensitivity and Ca<sup>2+</sup> dependence would suggest that activation of glutamatergic cells within the slice by 5-HT had led to an impulse flow-dependent release of glutamate. However, several lines of evidence argue against this conventional interpretation. First, rarely were neurons within the confines of the brain slice induced to fire by bath application of 5-HT. Second, none of the pyramidal cells (a potential source of intracortical excitatory inputs) was depolarized sufficiently by 5-HT as recorded under our conditions to reach the threshold for firing. Third, EPSCs can be induced by the microiontophoresis of 5-HT onto “hot spots” along the trajectory of apical dendrites of layer V pyramidal cells (118). Together, these experiments suggest that the increase in spontaneous EPSCs induced by 5-HT in neocortical pyramidal cells occurs through a focal action involving a Ca<sup>2+</sup>-dependent mechanism that is not based on an increase in impulse flow in excitatory afferents.

As an alternative to a conventional impulse flow-related mechanism, it has been hypothesized that the 5-HT-induced EPSCs result from an activation of the “asynchronous” release pathway (128). One of several distinguishing characteristics of this alternative mechanism of transmitter release is that Sr<sup>2+</sup> can substitute for Ca<sup>2+</sup> in the asynchronous, but not synchronous, release (129). This feature appears to be the result of the differential involvement of two isoforms of the calcium-sensing protein synaptotagmin in the two alternative release mechanisms (130). Consistent with this idea, Sr<sup>2+</sup> is highly effective in enabling 5-HT to induce an increase in the frequency of EPSCs in the absence of Ca<sup>2+</sup> (128).

Recently, it has been found that LSD and other hallucinogenic drugs, acting as partial agonists at 5-HT<sub>2A</sub> receptors, promote a late component of electrically evoked EPSPs (131). It is possible that this late component, rather than representing conventional polysynaptic transmission, is mediated through the mechanism of asynchronous transmitter release, possibly involving a release of intraterminal Ca<sup>2+</sup> stores via the phospholipase C, inositol triphosphate (IP<sub>3</sub>) pathway. An enhancement of asynchronous evoked EPSPs via 5-HT<sub>2A</sub> receptors would provide a possible synaptic mechanism for the hallucinogenic effects of these drugs. In contrast, 5-HT itself does not promote the late component of *electrically evoked* release except during the washout phase, presumably because of opposing actions at 5-HT<sub>1</sub> or other non-5-HT<sub>2A</sub> receptors (132). Figure 2.3 depicts the proposed location of various 5-HT-receptor subtypes and their interactions with other neurotransmitter receptors within cortical circuitry.



**FIGURE 2.3.** Modulation of excitatory and inhibitory transmission by multiple 5-hydroxytryptamine (5-HT) receptors in the cerebral cortex. 5-HT<sub>2A</sub> receptors are depicted as enhancing glutamate release from a glutamatergic terminal onto a layer V pyramidal cell; the same terminal is seen to be negatively modulated by various G<sub>i</sub>/G<sub>o</sub>-coupled receptors (e.g., μ-opiate, 5-HT<sub>1B</sub>, and mGluR II/III). In addition, 5-HT<sub>2A</sub> receptors are shown to have a direct postsynaptic excitatory effect that is opposed by postsynaptic 5-HT<sub>1A</sub> receptors. Finally, 5-HT<sub>2</sub> and 5-HT<sub>3</sub> receptors are shown on anatomically distinct GABAergic inputs to the somato-basilar and apical regions, respectively, of the pyramidal cell.

### 5-HT<sub>3</sub> Receptors

Excitatory responses to 5-HT have been found in various central neurons that have many of the characteristics of peripheral 5-HT<sub>3</sub> responses, including rapid onset and rapid desensitization, features typical of ligand-gated ion channels rather than G protein-coupled receptor responses (133, 134). In cultured NG108-15 cells, the permeation properties of the 5-HT<sub>3</sub> channel are indicative of a cation channel with relatively high permeability to Na<sup>+</sup> and K<sup>+</sup> and low permeability to Ca<sup>2+</sup> (134). A 5-HT-gated ion channel has been cloned that has physiologic and pharmacologic properties appropriate for a 5-HT<sub>3</sub> receptor (135). In the oocyte expression system, this receptor shows rapid desensitization and is blocked by 5-HT<sub>3</sub> antagonists (e.g., ICS 205–930 and MDL 72222). Its sequence homology with the nicotinic acetylcholine receptor (27%) and the β<sub>1</sub> subunit of the GABA<sub>A</sub> receptor (22%) indicates that this 5-HT<sub>3</sub>-receptor clone is a member of the ligand-gated ion channel superfamily. Typically, members of this superfamily are comprised of multiple subunits; however, only one 5-HT<sub>3</sub>-receptor subunit and an alternatively spliced variant have been cloned to date (136).

In hippocampus slices, 5-HT has been reported to increase spontaneous GABAergic IPSPs, most likely through a 5-HT<sub>3</sub> receptor-mediated excitation of inhibitory interneurons; these responses also show fading with time (137, 138). A similar 5-HT<sub>3</sub> receptor-mediated induction of IPSCs has been reported in the neocortex (117). Whole-cell patch-clamp recordings have confirmed a direct 5-HT<sub>3</sub> receptor-mediated excitatory effect on hippocampal interneurons independent of G-protein activation (139). Although fast, rapidly inactivating excitation has generally become accepted as characteristic of 5-HT<sub>3</sub> receptors, nondesensitizing responses have also been reported. In dorsal root ganglion cells, a relatively rapid but noninactivating depolarizing response has been described that has a 5-HT<sub>3</sub> pharmacologic profile (140). In neurons of nucleus tractus solitarius brain slices, a postsynaptic depolarizing response to 5-HT<sub>3</sub> agonists has been observed that is not rapidly desensitizing (141). In addition to these postsynaptic effects, a 5-HT<sub>3</sub> receptor-mediated increase in Ca<sup>2+</sup> influx has been described in a subpopulation of striatal nerve terminals (142).

#### 5-HT<sub>4</sub>, 5-HT<sub>6</sub>, and 5-HT<sub>7</sub> Receptors

The first known protein G<sub>s</sub>-coupled 5-HT receptor, the 5-HT<sub>4</sub> receptor, was identified on the basis of pharmacologic and biochemical criteria (e.g., positive coupling of 5-HT responses to adenylyl cyclase) (9). Subsequently, a receptor with matching pharmacologic and other properties was cloned and found to be expressed in various regions of the brain (143). Two other 5-HT receptors positively coupled to adenylyl cyclase have been cloned. Because their pharmacology differed from that of the previously described 5-HT<sub>4</sub> site, they were designated as 5-HT<sub>6</sub> and 5-HT<sub>7</sub> receptors (144–146). At this time, electrophysiologic studies are available only for the 5-HT<sub>4</sub> and 5-HT<sub>7</sub> receptors and are described below.

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

Binding studies using a selective 5-HT<sub>4</sub> ligand indicate that 5-HT<sub>4</sub> receptors are present in several discrete regions of the mammalian brain, including the striatum, substantia nigra, olfactory tubercle, and hippocampus (147). Because these regions also express 5-HT<sub>4</sub>-receptor mRNA, it appears likely that the receptors function postsynaptically to mediate certain actions of 5-HT. The best studied of these regions is the hippocampus, in which both biochemical and electrophysiologic studies have provided a detailed picture of the actions of 5-HT at 5-HT<sub>4</sub> receptors. Electrophysiologic studies show that 5-HT<sub>4</sub> receptors mediate an inhibition of a calcium-activated potassium current that is responsible for the generation of a slow after-hyperpolarization in hippocampal pyramidal cells of the CA1 region (74,148,149). A suppression of the after-hyperpolarization would enhance

the ability of these cells to respond to excitatory inputs with robust spike activity.

#### 5-HT<sub>7</sub> Receptors

The circadian rhythm in mammals is set by a pacemaker located primarily in the suprachiasmatic nucleus of the hypothalamus. This pacemaker activity can be maintained in hypothalamic slices, in which suprachiasmatic neurons display diurnal changes in neuronal firing rate. Administration of 5-HT appears to produce a phase shift in this activity (150) by acting on a receptor that may be of the 5-HT<sub>7</sub> subtype (144). This shift is mediated by stimulation of adenylyl cyclase because it is mimicked by increasing intracellular cyclic adenosine monophosphate (cAMP) and blocked by inhibiting protein kinase A (151). However, the precise mechanism by which 5-HT<sub>7</sub> receptors act is not presently known because it is unclear whether suprachiasmatic neurons themselves express the 5-HT<sub>7</sub> receptors (144). Furthermore, the effect of 5-HT on the membrane properties of these cells has not been examined. 5-HT<sub>7</sub> receptor activation has been reported to inhibit GABA<sub>A</sub> currents on suprachiasmatic neurons in culture (152), but the relationship, if any, between these observations and 5-HT changes in circadian activity remains to be determined.

Another electrophysiologic effect that may be mediated through 5-HT receptors that are positively coupled to adenylyl cyclase is the enhancement of the hyperpolarizing-activated nonselective cationic current I<sub>h</sub>. The I<sub>h</sub> channels, which are homologous to cyclic nucleotide-gated channels in specialized sensory neurons, are positively modulated by cAMP (153,154). An increase in I<sub>h</sub> tends to prevent excessive hyperpolarization and increase neuronal excitability. In a number of regions of the brain, including the thalamus (155), prepositus hypoglossi (156), substantia nigra zona compacta (157), and hippocampus (158), 5-HT has been shown to enhance I<sub>h</sub> through a cAMP-dependent mechanism. Results of a pharmacologic analysis with multiple nonselective drugs suggested that the increase in I<sub>h</sub> induced by 5-HT in dorsal root ganglion cells is mediated by 5-HT<sub>7</sub> receptors (159). Recently, the first drug with selectivity toward the 5-HT<sub>7</sub> receptor was shown to block activation of adenylyl cyclase by 5-HT agonists in guinea pig hippocampus (33). The increasing availability of such selective drugs should greatly enhance the electrophysiologic evaluation of G<sub>s</sub>-coupled 5-HT receptors.

## INTRACELLULAR SIGNAL TRANSDUCTION PATHWAYS

### Multiple Signaling Pathways: G Proteins and Second Messengers

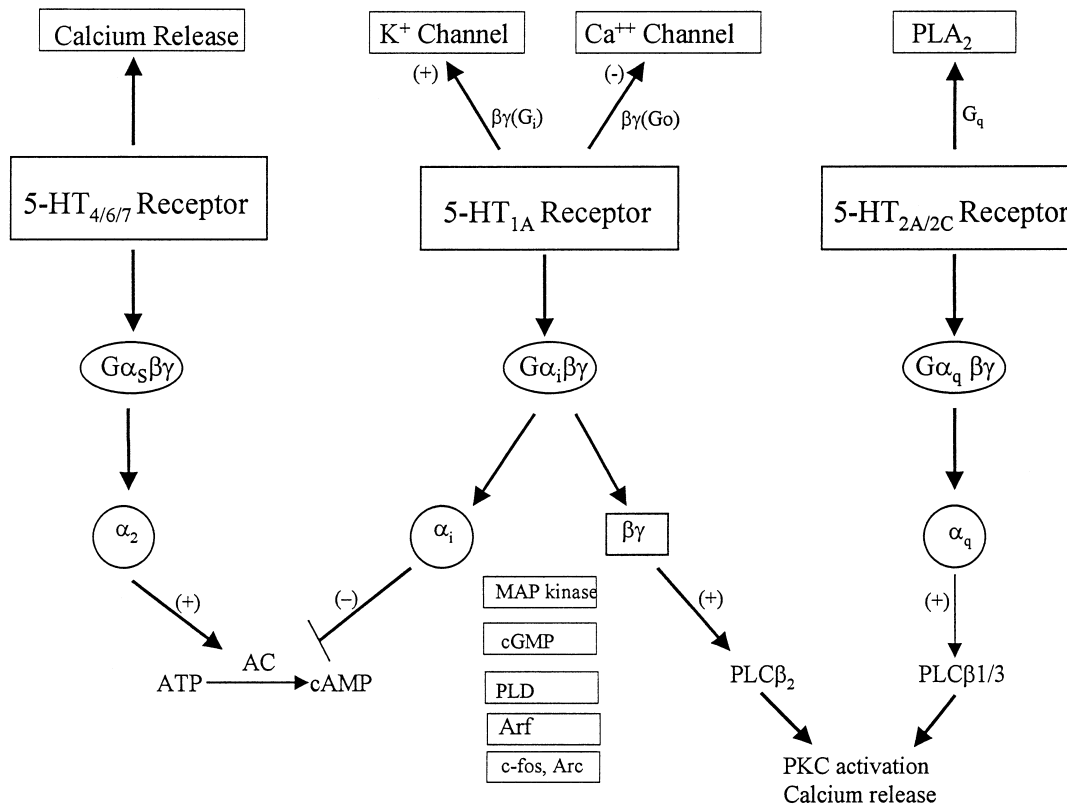
Multiple intracellular signaling pathways constitute a common theme for G protein-coupled receptors, and the 5-HT receptor family is not unique. Inhibition of adenylyl cyclase

was the first intracellular pathway to be described for  $G_{i/o}$  protein-coupled receptors, such as the 5-HT<sub>1A</sub> receptor. However, it is now clear that these receptors regulate multiple signaling pathways and effector molecules (Fig. 2.2), including activation of G protein-gated inwardly rectifying K<sup>+</sup> (GIRK) channels, inhibition of voltage-sensitive Ca<sup>2+</sup> channels, activation of phospholipase C $\beta$ , and activation of mitogen-activated protein kinase (see ref. 18 for review). Although all these signals are sensitive to pertussis toxin, so that  $G_{i/o}$  proteins are implicated, they may be mediated by distinct G protein complexes. For example, coupling to GIRK channels is mediated by  $\beta\gamma$  subunits released from  $G_i$  (and possibly  $G_o$ ) proteins, whereas inhibition of Ca<sup>2+</sup> channels is mediated by  $\beta\gamma$  subunits released from  $G_o$  proteins. The profile of signaling molecules varies from cell to cell, offering diverse signaling possibilities and contributing additional complexity. For example, 5-HT<sub>1A</sub> receptor activation of phospholipase C is cell-type dependent; this signal is mediated by G protein  $\beta\gamma$  subunits and thus requires the presence of a  $\beta\gamma$ -regulated phospholipase C isoform. The  $\beta\gamma$  subunits, generated by dissociation of the heterotrimeric  $G_i$  protein, also activate the type 2 isoform of adenylyl cyclase. This activation is conditional, dependent on the coactivation by  $G_{\alpha_s}$  (i.e.,  $G_{\alpha_i}$  potentiates the action of  $G_{\alpha_s}$ ). The obvious question is why the opposing actions of  $G_{\alpha_i}$  and  $G_{\beta\gamma}$  do not offset each other. The answer may lie in the details. In addition to the large family of G proteins (21  $\alpha$  subunits, 5  $\beta$  subunits, and 11  $\gamma$  subunits), the adenylyl cyclase family comprises at least nine members, each regulated by distinct inputs. Most of these molecules are found in the central nervous system. The G protein that contributes  $\beta\gamma$  activation of type 2 adenylyl cyclase is  $G_{\alpha_{i1}}$  or  $G_{\alpha_{i2}}$  heterotrimer (160), whereas all three  $G_{\alpha_i}$  subunits ( $\alpha_{i3} > \alpha_{i2} > \alpha_{i1}$ ) have the ability to inhibit adenylyl cyclase types 5 and 6 (161). Thus, in cells in the brain in which  $G_{\alpha_{i1}}$  or  $G_{\alpha_{i2}}$  heterotrimer is coexpressed with type 2 adenylyl cyclase, 5-HT<sub>1A</sub>-receptor activation may potentiate  $G_s$ -mediated increases in cAMP. This type of interaction has been shown to occur in brain, in which  $G_i$ -linked receptors enhance  $\beta$ -adrenergic responses (162); a similar interaction may take place in cells that coexpress a 5-HT<sub>1A</sub> receptor family member with one of the 5-HT receptors (5-HT<sub>4</sub>, 5-HT<sub>6</sub>, or 5-HT<sub>7</sub>) linked to activation of adenylyl cyclase.

Although a 5-HT receptor-mediated increase in cAMP formation in superior colliculus was one of the earliest second messenger pathways defined in brain, the 5-HT<sub>4</sub> receptor was one of the last 5-HT receptors to be cloned (143). This receptor and the 5-HT<sub>6</sub> and 5-HT<sub>7</sub> receptors have in common the ability to activate adenylyl cyclase via  $G_{\alpha_s}$  (Fig. 2.2). In transfected cells, the 5-HT<sub>6</sub> receptor couples to adenylyl cyclase type 5, the typical  $G_{\alpha_s}$ -sensitive isoform (163). In contrast, the 5-HT<sub>7</sub> receptor increases intracellular calcium, which activates calmodulin-stimulated adenylyl cyclase type 1 or 8. A recent characterization of rat hippo-

campal homogenates suggests that both the 5-HT<sub>4</sub> and 5-HT<sub>7</sub> receptors are involved in cAMP formation (adenylyl cyclase isoform unknown) in the hippocampus (164). Interestingly, the 5-HT<sub>1A</sub> receptor produces a slight increase in cAMP formation, perhaps reflecting  $G_{\beta\gamma}$  potentiation of  $G_s$  activation of adenylyl cyclase type 2 mediated by the 5-HT<sub>4</sub> or 5-HT<sub>7</sub> receptor.

Receptors that couple to the  $G_q$  family members ( $G_q$ ,  $G_{11}$ ,  $G_{14}$ , and  $G_{15/16}$ ) activate phospholipase C in a pertussis toxin-insensitive manner. Activation of phospholipase C was the first signal transduction mechanism identified for the 5-HT<sub>2</sub>-receptor family and is essentially universal. This probably reflects the wide distribution of  $G_{q/11}$  and the functional redundancy of these two G proteins. The 5-HT<sub>2C</sub> receptor has been shown to couple in a pertussis toxin-sensitive manner to  $G_{i/o}$  in *Xenopus* oocytes (e.g., see ref. 165) and in some transfected cell lines (166). In contrast, recent evidence suggests that phospholipase C activation in a native setting (choroid plexus) is mediated entirely by  $G_{q/11}$  coupling (167). Coupling of the 5-HT<sub>2C</sub> receptor to  $G_{13}$  with subsequent cytoskeletal rearrangement has been recently described in a transfected cell line (168). Extensive evidence suggests that 5-HT<sub>2A</sub> and 5-HT<sub>2C</sub> receptors couple to other effector pathways, in addition to phospholipase C (Fig. 2.4). Phospholipase A<sub>2</sub> is a well-characterized independent signal transduction pathway that leads to arachidonic acid, with subsequent prostaglandin and leukotriene formation (169). 5-HT<sub>2A</sub>-receptor activation of mitogen-activated protein kinase has been extensively characterized in vascular smooth muscle and is also thought to be independent of phospholipase C activation (170,171). The 5-HT<sub>2A</sub> receptor increases phospholipase D activity via a small G-protein ARF (adenosine diphosphate ribosylation factor) pathway, with protein kinase C activation being the principal consequence (172). 5-HT<sub>2A</sub> receptors differentially regulate brain-derived neurotrophic factor in hippocampus and cortex and play a role in stress-induced down-regulation of brain-derived neurotrophic factor expression in hippocampus (173,174). In addition, a 5-HT<sub>2A</sub> receptor-mediated increase in transforming growth factor- $\alpha_1$ , secondary to protein kinase C activation, has been described (175). The 5-HT<sub>2A</sub> and 5-HT<sub>2C</sub> receptors elicit region-specific increases in immediate early genes *c-fos* and *Arc* in rat brain (176), which are likely downstream of phospholipase C activation. Extensive, complex cross-talk between the 5-HT<sub>2A</sub> and 5-HT<sub>2B</sub> receptor and the 5-HT<sub>1B/D</sub> receptor has been demonstrated in immortalized serotonergic cells, in which the 5-HT<sub>2B</sub> receptor, via a phospholipase A<sub>2</sub> product, attenuates 5-HT<sub>1B/D</sub> receptor-mediated adenylyl cyclase inhibition (177). Coactivation of the 5-HT<sub>2A</sub> receptor blocks this interaction by an unknown mechanism. These examples of parallel, interacting, and converging intracellular signaling pathways illustrate the complexity of receptor signaling, even within a single receptor subclass.



**FIGURE 2.4.** Examples of potential converging and interacting signaling pathways for 5-hydroxytryptamine-receptor subtypes. This figure illustrates only a few of the nearly unlimited possibilities, depending on the cell phenotype. Also listed are additional effectors activated by one or another of these receptors with pathways of activation that have not yet been determined.

## Physiologic Correlates

In general, the electrophysiologic effects of 5-HT correspond well to the G-protein and second messenger coupling of the various receptor subtypes. The  $G_i/G_o$ -coupled 5-HT<sub>1</sub> receptors generally mediate inhibitory effects on neuronal firing through an opening of inwardly rectifying  $K^+$  channels or a closing of voltage-gated  $Ca^{2+}$  channels. Inhibitions mediated by 5-HT<sub>1</sub> receptors have been observed in neurons located in diverse regions of the central nervous system, ranging from pyramidal cells of the cerebral cortex and hippocampus to serotonergic neurons of the brainstem raphe nuclei. The  $G_{q/11}$ -coupled 5-HT<sub>2</sub> family of receptors generally mediates slow excitatory effects through a decrease in  $K^+$  conductance or an increase in nonselective cation conductance. Slow excitatory effects mediated by 5-HT<sub>2</sub> receptors have been observed in a number of regions, including the spinal cord and brainstem (e.g., motoneurons), subcortical regions (e.g., nucleus accumbens), and cerebral cortex, where these receptors are most concentrated. The 5-HT<sub>3</sub> receptors, which are ligand-gated channels with structural homology to nicotinic cholinergic receptors, mediate fast excitatory effects of 5-HT. Specific examples are given

below for 5-HT<sub>1</sub>, 5-HT<sub>2</sub>, and 5-HT<sub>4</sub> receptors, for which intracellular transduction pathways have been studied most intensively.

## 5-HT<sub>1</sub> Receptors

The opening of  $K^+$  channels via 5-HT<sub>1A</sub> receptors in dorsal raphe neurons is mediated by pertussis toxin-sensitive G proteins (178,179). The molecular mechanisms underlying the opening of  $K^+$  channels are most likely common to all neurotransmitter receptors that couple through the  $G_i/G_o$  family of G proteins. As in the dorsal raphe, these receptors activate a pertussis toxin-sensitive G protein that couples to the opening of inwardly rectifying  $K^+$  channels through a membrane-delimited pathway (74,180). It is widely accepted that the  $\beta\gamma$  rather than  $\alpha$  subunits regulate the channels (181–183). The effector mechanism that ultimately mediates the inhibitory effect signaled by 5-HT<sub>1A</sub> receptors is the inwardly rectifying  $K^+$  channel. Interestingly, at least one of the potassium  $K^+$  subunits identified in heart, GIRK-1, is expressed at high levels in hippocampus (184), which suggests that it might be involved in mediating the 5-HT<sub>1A</sub> receptor-induced hyperpolarization in this region.

Consistent with this possibility, the  $K^+$  current activated by 5-HT<sub>1A</sub> receptors in the CA1 region does show the characteristic signature of this potassium channel family—namely, inward rectification (74).

### 5-HT<sub>2</sub> Receptors

The role of G proteins in mediating the 5-HT<sub>2</sub>-induced slow inward current that results from  $K^+$  channel closure has been evaluated in facial motoneurons by using the hydrolysis-resistant guanine nucleotide analogues GTP $\gamma$ S and GDP $\beta$ S (185). The 5-HT-induced inward current becomes largely irreversible in the presence of intracellular GTP $\gamma$ S. Mediation by G proteins is also suggested by the fact that the inward current is reduced by intracellular GDP $\beta$ S, which prevents G-protein activation. Although the identity of the G protein(s) mediating the electrophysiologic responses has not yet been determined directly, the 5-HT<sub>2</sub> family of receptors is known to be coupled to phospholipase C. Thus, a member of the G<sub>q/11</sub> family may be involved because the latter can directly activate phospholipase C (186).

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

Initially, it was shown that 5-HT suppresses a calcium-activated potassium current that is responsible for the generation of a slow after-hyperpolarization in hippocampus pyramidal cells of the CA1 region (see above). Subsequent studies have implicated 5-HT<sub>4</sub> receptors, acting via cAMP and protein kinase A, in mediating this action (187). A similar activation of a cAMP-dependent protein kinase has been implicated in the suppression of a voltage-activated  $K^+$  current in cultured neurons from the superior colliculus (188). More recently, it has been shown that 5-HT<sub>4</sub> receptors reduce after-hyperpolarization in hippocampus pyramidal cells by inhibiting calcium-induced calcium release from intracellular stores (189).

## Pharmacologic Significance

The pharmacologic significance of a single receptor regulating multiple signaling pathways is only just beginning to be defined. The most explicit studies of promiscuous coupling of receptors to multiple G-protein signaling pathways have involved transfection of a recombinant receptor into various cell models that do not normally express the receptor. Powerful genetic strategies involving antisense techniques, overexpression of signaling molecules, and expression of constitutively active and dominant negative mutants have exposed a multitude of fascinating possibilities for a single receptor to sculpt multiple signals depending on the properties of the cell. In addition, theoretical arguments (190) and experimental evidence (191–193) have appeared in support of the novel concept of agonist-directed traffick-

ing of the intracellular signal. This model proposes that when a single receptor interacts with multiple signaling pathways, the pattern of intracellular signaling may differ depending on the agonist. Although the mechanism of agonist-specified signaling is not known, one possibility is that different agonists promote distinct receptor conformations, thereby exposing interfacial domains with altered protein–protein interaction properties. All these studies in artificial conditions tell us only what can occur, not what does occur *in vivo*. Techniques for studying the role of multiple signaling pathways in native preparations are needed to tease out the significance of the various signaling molecules in normal physiology and in pathologic states. Transgenic and knockout strategies have some utility; however, targeting signaling molecules will have a multitude of unwanted consequences because of their universal role in cell physiology. Another strategy was recently described that has significant potential for teasing out signaling pathways downstream of receptor activation (167). Synthetic blocking peptides targeting specific protein–protein interactions in a signaling pathway are rendered membrane-permeable by a novel conjugation reaction, so that the function of a particular signaling step in native systems can be defined.

## BEHAVIORAL CORRELATES

### 5-HT Neuronal Activity and Behavioral State

In a variety of mammalian species, serotonergic neurons of the raphe nuclei have been found to have a slow, tonic pattern of firing (approximately one to two spikes per second). The maintenance of rhythmic firing under a wide variety of conditions has suggested that serotonergic neurons possess intrinsic tonic pacemaker mechanisms. Intracellular recordings from dorsal raphe neurons show that spikes arise from gradual depolarizing ramps (pacemaker potentials) rather than synaptic potentials. The pacemaker rhythm of serotonergic neurons results from a complex interplay of intrinsic ionic currents (e.g., a voltage-dependent transient outward potassium current, a low-threshold inward calcium current, and a calcium-activated outward potassium current) (194). Also modulating the activity of serotonergic neurons are various neurotransmitters, including norepinephrine and 5-HT itself. Norepinephrine, acting via  $\alpha_1$  adrenoceptors, accelerates pacemaker activity of serotonergic neurons by closing potassium channels. Conversely, 5-HT itself, acting on 5-HT<sub>1A</sub> autoreceptors, opposes excessive activity of serotonergic neurons.

The highly regulated pacemaker activity of serotonergic neurons suggests that the 5-HT system serves an important homeostatic function. Through its effects on neuronal excitability in diverse regions of the brain and spinal cord, the serotonergic system is in a strategic position to coordinate complex sensory and motor patterns during different

behavioral states. Recordings from serotonergic neurons in unanesthetized animals have shown that activity is highest during periods of waking arousal, reduced in quiet waking, reduced further in slow-wave sleep, and absent during REM (dream) sleep (195). It can be hypothesized that the function of the 5-HT system, by its coordinated fluctuations in activity, is to promote a given behavioral state. This concept is illustrated in the following scenario. When serotonergic neurons are in a tonic firing mode, the following conditions would prevail: (a) Motoneurons would be in a relatively depolarized, excitable state (via 5-HT<sub>2</sub> receptors) and thus receptive to the initiation of movement; (b) neurons of the nucleus reticularis thalami would be in a depolarized, single-spike mode (via 5-HT<sub>2</sub> receptors) and thus conducive to thalamocortical sensory information transfer (105,155); (c) GABAergic neurons of the septohippocampal pathway would be activated (in part via 5-HT<sub>2A</sub> receptors), potentially enhancing long-term potentiation by inhibiting GABAergic neurons of the hippocampus (106,196); (d) neurons of the laterodorsal tegmental nucleus would be hyperpolarized (via 5-HT<sub>1</sub> receptors) and therefore not able to generate the bursting activity of REM sleep (69–71). Conversely, with a reduction in serotonergic activity during various stages of sleep, the above conditions would switch such that motoneurons would become less excitable, thalamocortical sensory information transfer would be diminished, hippocampal function would be reduced, and sleep spindles and pontogeniculo–occipital (PGO) waves would emerge.

## Molecular Genetics (Including Genetic Polymorphisms)

### 5-HT-Receptor/Transporter Knockouts

New drugs are beginning to appear that show considerable selectivity for a particular serotonin receptor subtype; however, many are not yet readily available to the general scientific community. Genetically modified mice that fail to express a specific receptor provide a powerful means to complement pharmacologic tools for evaluating the behavioral consequences of a particular serotonin-receptor protein (see ref. 197 for review). The first 5-HT-receptor knockout mouse was described in 1994 (198), in which the 5-HT<sub>1B</sub> receptor was eliminated by homologous recombination technique. These original studies showed markedly enhanced aggression in 5-HT<sub>1B</sub>-receptor knockout mice. Since then, altered responses to drugs of abuse, including enhanced alcohol consumption (199) and sensitization to cocaine (200), in addition to impaired paired-pulse inhibition (201) and paradoxical sleep (202), have been shown to be prominent phenotypic traits. In 1995, a “knockout” mouse line expressing a mutant, nonfunctional 5-HT<sub>2C</sub> receptor was described (203). Subsequently, enhanced seizure susceptibility (204), obesity and late-onset diabetes (205),

and a specific deficit in dentate gyrus long-term potentiation (206) have been reported. Mouse lines have recently been generated that are null for other important 5-HT-related molecules; these including the 5-HT<sub>1A</sub> receptor, which is associated with enhanced anxiety (207–209), the serotonin transporter, with enhanced cocaine sensitivity (210,211), and the 5-HT<sub>5A</sub> receptor, with reduced sensitivity to LSD (212). Although monoamine oxidase A-null mice have general alterations in biogenic amine dynamics, evidence suggests that the enhanced levels of 5-HT found in these mice are associated with neurodevelopmental abnormalities (213, 214). Innovative technologies such as inducible, conditional knockouts, which have the potential for temporally and spatially controlling gene manipulation, hold great promise for the future. This is illustrated in a recent study in which localized rescue of knocked-out genes was used to study the differential sorting of the 5-HT<sub>1A</sub> and 5-HT<sub>1B</sub> receptor in striatal neurons (215). In these transgenic mice, but not transfected neurons in culture, reproduction of the normal targeting of the 5-HT<sub>1B</sub> receptor to axon terminals set the stage for mutagenesis studies of molecular determinants of receptor targeting to axon terminals *in vivo*.

### Genetic Polymorphisms

Molecules involved in brain 5-HT pathways have been favorite targets for candidate gene studies, and the number of publications dealing with genetic variations in 5-HT systems has increased dramatically during the past few years. Recent population studies have probed for single nucleotide polymorphisms in synthetic enzymes, inactivation molecules, and receptors for 5-HT. The list of human diseases studied is extensive and includes obsessive-compulsive disorder, major depression, bipolar depression, schizophrenia, Alzheimer’s disease, eating disorders, anxiety, neuroticism, fibromyalgia, alcoholism, suicide, homicide, substance abuse, pathologic gambling, and responses to psychotherapeutic agents. Despite the abundance of publications, no definitive, reproducible links between allelic variants of 5-HT-related molecules have been found in human populations with behavioral disorders or brain diseases. More often than not, results are not reproducible from study to study, in large part because of the heterogeneous nature of psychiatric diseases, the absence of a specific diagnostic laboratory test, and the modest numbers of patients in many studies. The most extensively studied genetic polymorphism in a 5-HT-related molecule is the insertion/deletion polymorphism in the promoter region of the 5-HT transporter gene (216). These variable-length polymorphisms are biologically significant because *in vitro* studies have shown that the short form reduces the expression of transporter mRNA, with subsequently reduced uptake capacity (217). Although many studies suggest that the short form is associated with affective disorders, others have failed to replicate these findings (218).

Some commonly studied polymorphisms, such as the C103T variant in the 5-HT<sub>2A</sub> receptor, are silent (i.e., do not change the genetic code), whereas other polymorphisms, such as the 5-HT<sub>2C</sub> receptor Cys23Ser allele (219), produce mutant proteins with no apparent alterations in functional properties. The clinical importance of such a subtle genetic variant may require analysis of other related genes in tandem. Methods for detecting genetic polymorphisms are advancing rapidly and now allow simultaneous genotyping of several nucleotide polymorphisms; for example, a method was recently described to detect multiple single-nucleotide polymorphisms of 5-HT-related genes (220).

## OVERVIEW AND CONCLUSIONS

This review has emphasized recent developments in molecular, transductional, and cellular aspects of the 5-HT system. Molecular topics that were hardly mentioned in the previous edition of this book include RNA editing, post-translational processing, genetic polymorphisms, and the use of selective 5-HT-receptor and transporter gene knockouts. Notable developments in cellular physiology since the previous edition include growing numbers of studies on the more recently cloned 5-HT-receptor subtypes (e.g., 5-HT<sub>3-7</sub>) and refinements in the analysis of 5-HT<sub>1-</sub> and 5-HT<sub>2-</sub>receptor function. Examples of the latter include the following: (a) the recognition that the well-known 5-HT<sub>1A</sub> autoreceptor feedback regulation of 5-HT neurons occurs within the context of a complex set of local and long-loop regulatory circuits; (b) the finding that 5-HT<sub>2</sub> receptors have a dramatic influence on cortical information processing, which has allowed new insights into the mechanism of action of hallucinogenic and atypical antipsychotic drugs. In turn, advances in molecular and cellular research on individual receptor subtypes have provided new experimental tools for the behavioral analysis of the 5-HT system (e.g., pharmacologic agents with more precisely defined actions and gene knockouts). The question remains of whether the diverse cellular and molecular actions of 5-HT mediated by the various receptor subtypes can be incorporated into a holistic scheme that can define the overall function of the 5-HT system. Selected examples have been given of how the 5-HT system can be seen as modulating, in a complex but coordinated fashion, a number of motor, sensory, and other systems to promote a given behavioral state or function. The recent molecular and cellular advances, by enabling a more comprehensive analysis of the elementary actions of individual 5-HT-receptor subtypes, have set the stage for a more precise analysis of overall function.

## ACKNOWLEDGMENTS

This work was supported by grants from the National Institute of Mental Health, the National Institute on Drug Abuse, and the state of Connecticut.

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