Review

Signaling at G-protein-coupled serotonin receptors: recent advances and future research directions

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The broadly distributed monoaminergic neurotransmitter serotonin (5-hydroxytryptamine, 5-HT) exerts its actions via 14 classes of receptor. With the exception of 5-HT_3 receptors, which gate a cation-permeable ion channel, all 5-HT receptors are coupled to G proteins. The core features of transduction via 5-HT receptors are well established, but much still remains to be learned, in particular, with regard to native populations in the brain. In this article, we survey the current knowledge of cellular signaling at G-protein-coupled 5-HT receptors and focus on several novel (and surprising) insights that have emerged over the past few years. We also highlight several promising directions for future research that should improve the understanding of serotonin signaling and ultimately permit its therapeutic exploitation in the control of central nervous system disorders. In view of the diversity of transduction mechanisms engaged by 5-HT, much of this discussion is relevant to other classes of G-protein-coupled receptors.

Introduction

The phylogenetically ancient monoamine 5-hydroxytryptamine (5-HT), which is found in organisms as diverse as barnacles, bumble-bees, bears and bower-birds, fulfills a broad and species-specific role in the control of many vital functions.

In humans, 5-HT is derived from dietary tryptophan, which is transformed into 5-HT in the brain mainly by the neuron-specific '2' isoform of tryptophan hydroxylase (Figure 1). Its actions are terminated by transporter-mediated reuptake into neurons, leading to catabolism by monoamine oxidase (Figure 1). 5-HT functions via 14 classes of receptor, which are all present (and differentially distributed) in the central nervous system (CNS), including the frontal cortex, hippocampus, amygdala, striatum, hypothalamus and dorsal horn [1–4]. Through actions at these multiple classes of receptor, 5-HT controls almost any core CNS function one might care to mention, such as mood, cognition, sleep, pain, motor function and endocrine secretion. Correspondingly, a disruption of serotonergic transmission is implicated in the pathogenesis of depression, anxiety, schizophrenia and chronic pain, and many agents used for their treatment function, at least partially, via serotonergic mechanisms [1,2].

An improvement of serotonergic therapeutics necessitates a better understanding both of the functional significance of multiple classes of 5-HT receptor and of their actions at the cellular level. Indeed, although the basic characteristics of serotonin signaling are now familiar, most studies have been performed on recombinant receptors individually expressed in non-neuronal cell lines [3–5] (Box 1 and Figure 1). As emphasized later, we are still woefully ignorant of how cerebral 5-HT receptors operate in real life: alone and in interaction with other sites, under physiological and pathological conditions, and in response to therapy.

The term 'signaling' is, in a sense, open ended. Ultimately, signaling is translated into changes of mood and behavior, whereas alterations in electrical activity and gene expression are relevant endpoints at the cellular level. However, integration of such information would render this review inordinately diffuse. Thus, the present article focuses on (i) the influence of G-protein-coupled 5-HT receptors upon soluble second messengers and (ii) the remarkable diversity of serotonergic signaling in the CNS. Several recently discovered insights into the cellular actions of 5-HT are highlighted, together with other novel themes likely to animate research in this field over the coming years.

Recent developments: novel aspects of serotonergic signaling

New signaling pathways recruited by G-protein-coupled serotonin receptors

The principal signaling mechanisms recruited by 5-HT receptors are outlined in Box 1 and Figure 1, and the influence of post-transcriptional modification of 5-HT receptors upon transduction is summarized in Box 2. However, there are many variations on the core themes of serotonin transduction (Table 1), and the following recent observations are of special interest.

5-HT_6 receptors are well known to recruit $G\alpha_s$ and adenylyl cyclase (AC), but their cellular pharmacology was recently enlivened by the finding that their activation triggers phosphorylation of Fyn, a member of the Src family of non-receptor tyrosine kinases. Fyn, in turn, activates the key intracellular modulator, extracellular regulated kinase

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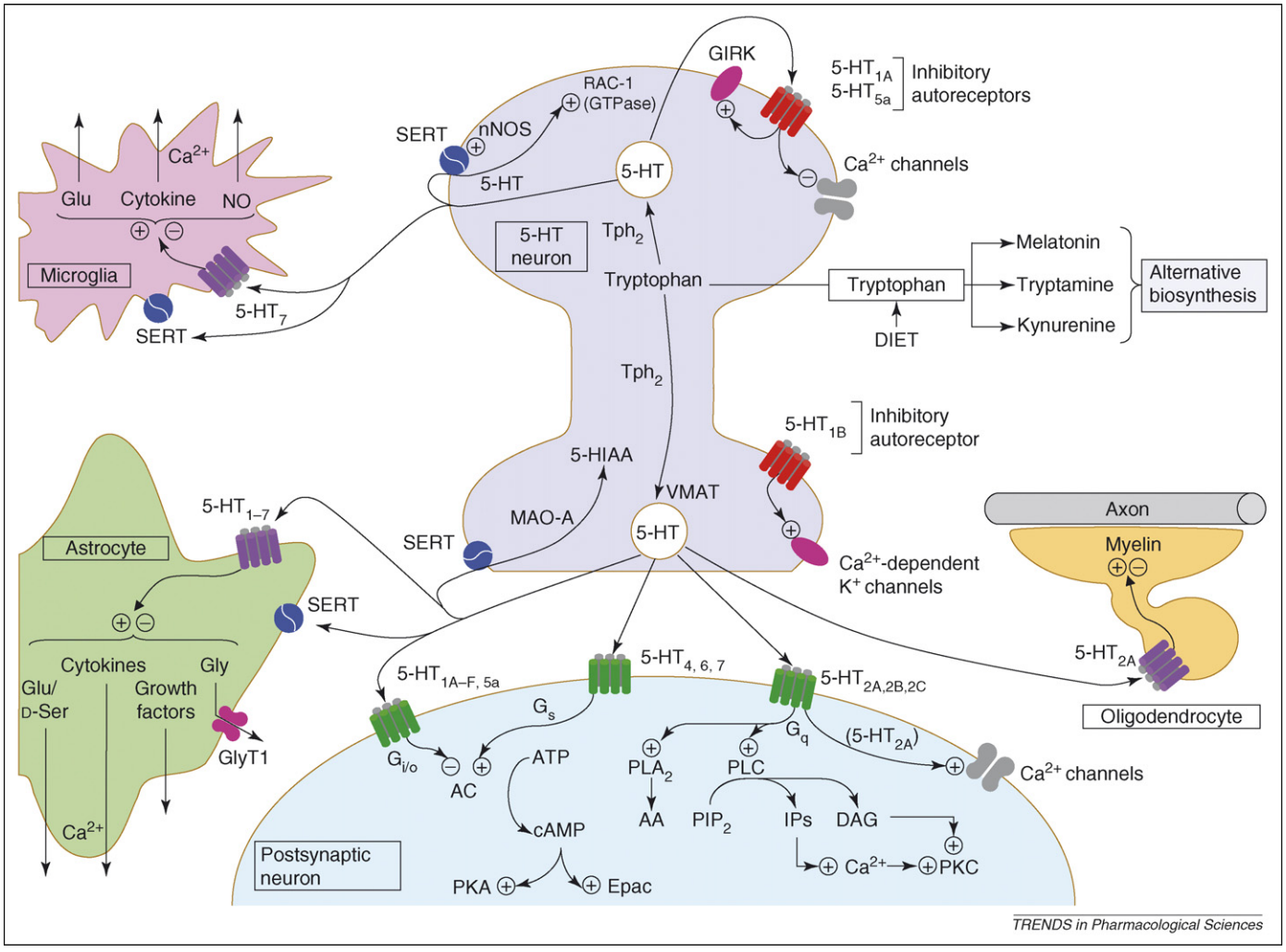

Figure 1. An integrated view of signaling at serotonergic neurons. 5-HT is derived from tryptophan by an action of tryptophan hydroxylase 2 (Tph_2), and it is deactivated by monoamine oxidase (MAO) A after release and reuptake via 5-HT transporters (SERT). Generation of 5-HT from tryptophan is an alternative to its conversion into melatonin (in the pineal gland), tryptamine (in neurons) and kynurenine (in astrocytes), which likewise function as neuromodulators in the brain. 5-HT receptors are localized both pre- and post-synaptically to serotonergic neurons, but all subtypes are not necessarily co-localized at the same postsynaptic location. 5-HT_{1A} and 5-HT_{1B} inhibitory autoreceptors are localized on cell bodies and terminals, respectively, and 5-HT_{5a} autoreceptors might also be present on the former. Characterization of non-neuronal 5-HT receptors is far from complete. (Note that ligand-gated ion-channel 5-HT_3 receptors, not considered herein, are present postsynaptically both on neurons and on non-neuronal cells.) The major modes of transduction are shown at the postsynaptic level and many receptors converge on specific signaling pathways. Moreover, individual subtypes recruit multiple cascades, for example 5-HT_{2A} receptors couple to Ca^{2+} channels, PLC and PLA_2. In addition, the GTPase exchange factor Epac is a recently identified downstream target of cAMP. Ion currents are an important mode for autoreceptor-feedback inhibition of serotonergic transmission. Serotonergic neurons also reveal two new modes of signaling: direct activation of neuronal nitric oxide (NO) synthase (nNOS) by SERTs and activation of a small G protein–GTPase, Rac-1, by 5-HT itself. Coupling patterns in non-neuronal cells show similarities and differences to neurons, but await further characterization. Abbreviations: 5-HIAA, 5-hydroxyindole amino acid; DAG, diacylglycerol; Gly, glycine; GlyT1, glycine transporter; IP, inositol phosphate; PIP_2, phosphoinositol bisphosphate; D-Ser, D-serine; VMAT, vesicular monoamine transporter.

(ERK)1/2, via a classical Ras–Raf1–MEK (mitogen-activated protein kinase kinase) cascade [6]. Reciprocally, in a form of positive feedback, binding of Fyn to the C terminus of 5-HT_6 receptors increases their cell-surface expression.

Further boosting interest in G_s-coupled 5-HT receptors, it was recently found that generation of cAMP activates the GTPase exchange factor, Epac (exchange protein directly activated by cAMP); stimulation of AC can, then, either recruit Epac and/or activate protein kinase (PK) A (Figure 1). Accordingly, G_s- and AC-coupled 5-HT_7 receptors induce ERK1/2 both via PKA and the Ras–Raf1–MEK cascade and via Epac, which initiates an alternative route to ERK phosphorylation. Furthermore, in primary cultures of cortical neurons, engagement of Epac by 5-HT_4 receptors activates α-secretases; these subsequently generate soluble amyloid precursor protein, which has neuroprotective and memory-enhancing properties [7].

An additional new twist to serotonergic signaling comprises the amiloride-sensitive Na^+/H^+ exchanger (NHE), which is omnipresent in membranes of mammalian cells (including serotonergic neurons in brain), where it regulates cellular volume and intracellular pH. NHE is G-protein activated by 5-HT_{1A} and 5-HT_{2A} receptors, yet (at least in the gut), inhibited by 5-HT_4 receptors [8–10]. 5-HT_{1A} and 5-HT_{2A} receptors exert an additional mode of facilitatory control via phosphorylation of the tyrosine kinase, Janus kinase-2 (JAK2), which, in turn, stimulates Ca^{2+}-calmodulin binding to NHE [8]. Supporting the functional pertinence of NHE, its inactivation exacerbates vulnerability of serotonergic terminals to the recreational drug ecstasy [11].

Most remarkably, as discussed in the following paragraph, recent studies have unveiled roles for the 5-HT transporter (SERT) and for 5-HT itself in signaling.

Box 1. Current status of serotonin signaling

Cloning of 5-HT receptors led to the recognition of several families differentially coupled via specific G proteins to 'prototypical' transduction pathways [3–5] (see Table 1 in the main text). All (intronless) 5-HT_1 receptors negatively couple to adenylyl cyclase (AC) via $G_{i/o}$, whereas 5-HT_4, 5-HT_6 and 5-HT_7 receptors stimulate AC through G_s. Intron-rich 5-HT_4 receptors possess up to 10 C-terminal splice variants, but all share (despite some subtle differences) similar coupling profiles, including recruitment of G_s and AC. The same applies to the four C-terminal splice variants of 5-HT_7 receptors, although patterns of internalization differ. By contrast, the 5-HT_2 triplets (A, B and C) share recruitment of PLC via $G_{q/11}$ as their primordial mode of signaling. Uniquely, 5-HT_{2C} receptors display adenosine-to-inosine mRNA editing, which yields 15–20 isoforms possessing contrasting amino acid sequences (see Figure 2 in the main text). Non-edited, partially edited and fully edited isoforms are differentially distributed throughout the brain [21,31]. As pointed out later, editing has important consequences for signaling. No cardinal pathway is, as yet, recognized for 5-HT_{5a} receptors, but it seems that they primarily couple to $G_{i/o}$ [4,82].

Many effects of 5-HT receptors on ion currents are secondary to other transduction pathways [3,4] (see Table 1 in the main text). However, 5-HT_{1A} (and, perhaps, 5-HT_{5a}) autoreceptors directly recruit Kir.3 K^+ channels (GIRKs) (and ERK1/2) via $G_{\beta\gamma}$ subunits, leading to inhibition of raphe-localized serotonergic perikarya (see Figure 1 in the main text). This suppression of excitability is complemented by inhibition of (N and P/Q-type) Ca^{2+} channels [3,51]. Conversely, potentiation of Ca^{2+}-dependent K^+ channels contributes to the inhibitory influence of 5-HT_{1B} (and, possibly, 5-HT_{1D}) autoreceptors on serotonergic terminals and, hence, 5-HT release (see Figure 1 in the main text).

Innumerable variations on the above themes have been reported, as summarized in Table 1 (see the main text), and individual classes of receptor access multiple signals. For example, 5-HT_{2C} receptors function not only via PLC but also via PLA_2 and PLD [21,83] (see Figure 2 in the main text). Furthermore, some pathways are recruited independently of G proteins. Generally speaking, a specific 5-HT-receptor subtype influences several G proteins and downstream messengers ('divergence'). Moreover, multiple 5-HT receptors collectively impact ('convergence') individual signals such as AC and ERK, in addition to transcription factors such as cAMP-response-element-binding protein (CREB) [2,5] (see Figure 3 in the main text).

Thus, there are no absolute distinctions between 5-HT receptors in their signaling modes, and no individual subtype possesses just one single transduction mechanism.

Box 2. Post-translational modification of 5-HT receptors: influence on signaling

5-HT receptors possess sites susceptible to post-translational modification, which can markedly modify signaling. In certain cases, prolonged stimulation of 5-HT receptors leads to their phosphorylation by G-protein-receptor kinases and/or by PKA and PKC. Phosphorylation of 5-HT receptors can also be triggered heterologously by other co-localized GPCRs, which likewise recruit these classes of kinase. Phosphorylation is important because it modifies cell-surface expression, coupling profiles and interactions with protein partners, usually leading to blunted signaling [52]. Intriguingly, activation of 5-HT receptors can also promote phosphorylation of Gα proteins, which further attenuates transduction; for example, sustained activation of 5-HT_{2A} receptors is accompanied by phosphorylation of $G_{q/11}$ [51,84]. Note also that regulators of G-protein signaling (RGSs) blunt transduction by binding to Gα proteins, which accelerates GTP hydrolysis [79]. Glycosylation (addition of an oligosaccharide) of 5-HT receptors might also be important, and *N*-asparagine glycosylation is a requirement for insertion of 5-HT_{5A} receptors into the plasma membrane and coupling to transduction mechanisms [82]. Palmitoylation is the attachment of palmitate via a thioester link to cysteine residues. Agonist-enhanced palmitoylation of 5-HT_4 receptors modulates spontaneous coupling to G_s and affects sensitivity to phosphorylation and internalization [85]. Furthermore, palmitoylation of 5-HT_{1A} receptors enhances their retention in plasma-membrane-localized lipid rafts, thereby favoring coupling to $G_{i/o}$ and AC [86].

Direct modulation of signaling by SERTs and by 5-HT itself

Activation of 5-HT receptors and of other classes of G-protein-coupled receptors (GPCRs) triggers the phosphorylation of SERTs by PKC and PKG or p38. These kinases decrease and enhance SERT membrane expression, respectively, and, correspondingly, they reduce and accelerate, respectively, the kinetics of 5-HT uptake [2,12]. Thus, signaling is indirectly modified by altered availability of 5-HT. However, providing a new dimension to the relationship between SERT and transduction SERTs were recently found to directly control the activity of neuronal nitric oxide synthase (nNOS) [13]. Thus, a proteomic approach showed that nNOS binds to the extreme C terminus of SERT and, in cells co-expressing SERT and nNOS, exposure to 5-HT generates nitric oxide (NO) and cGMP in a calmodulin-dependent manner. 5-HT-reuptake inhibitors block this action, showing that passage of 5-HT through SERT is necessary for nNOS recruitment. Reciprocally, association of nNOS with SERT restricts their membrane insertion, and 5-HT reuptake is enhanced in mice genetically lacking nNOS, demonstrating that this interaction occurs in the brain. Finally, NO also exerts feedback actions on SERT via activation of PKG [2].

A further revelation is that 5-HT itself can affect signaling. In platelets, SERT-mediated accumulation of 5-HT results in its 'transamidation' to small GTPases such as Rho-A and Rab-4 [14]. Transamidation is catalyzed by transglutaminases, a family of Ca^{2+}-dependent enzymes. This process of 'serotonylation' renders GTPases active, leading to α-granule exocytosis. Activation of 5-HT_{2A} receptors, which increases Ca^{2+} availability, synergizes with SERT to induce 5-HT transamidation [14]. Indicating that comparable events occur in brain, 5-HT_{2A} receptors enhance transamidation of 5-HT to another Rho-family GTPase, Rac-1, in cells derived from cortex [15] (Figure 1). Finally, transamidation participates in a negative loop that regulates 5-HT uptake [16]. Thus, exposure of platelets to 5-HT promotes association of the 5-HT-bound activated form of Rab-4 to the C-terminal domain of SERT, leading to its intracellular retention [16].

G-protein-dependent, ligand-directed signaling

At certain classes of GPCRs, agonists show contrasting signaling patterns. Some more efficaciously recruit one pathway versus another, whereas others display opposite profiles. These differences reflect induction (stabilization) of multiple conformational states, a phenomenon named 'ligand-directed trafficking or signaling' [17].

Accordingly, 5-HT_{1A}-receptor agonists show dissimilar efficacies at various isoforms of Gα protein (G_{i2} versus G_{i3}, G_i versus G_s) [3,18] and at specific ion currents (Kir.3 K^+ channels, known as GIRKs, versus smooth inward-current channels, known as I_{smooth}) [19]. Furthermore, distinctive actions of agonists at pre- versus postsynaptic 5-HT_{1A}

Table 1. Coupling patterns of multiple classes of 5-HT receptor[a]

Receptor (variant)	Population	Principal G proteins	Principal signals	Other G proteins	Other direct signals	Downstream signal (mediator)[b]
5-HT_{1A}	Recombinant	$G_{i/o}$ (G_{i1}, G_{i2}, G_{i3}, G_o)	AC and PKA (−) gK^+ (GIRK) (+) gCa^{2+} (N, P/Q) (−)	G_z	PLC, Ca^{2+} and PKC (+) PLA_2 and AA (+) AC II (+)	pERK (Ras, PI3K) (+) pAkt (PI3K) (+) JNK (Rho) (+) [87] NHE (Jak2 and CaM) (+) I_{smooth} (+)
	Endogenous (brain)	G_i (DRN) $G_o > G_i$ (Cx, Hip, Hyp)	AC and PKA (Cx, Hip) (−) gK^+ (GIRK) (DRN) (+) gCa^{2+} (N, P/Q) (DRN) (−)	G_z (Hyp, Hip)	PLC (Hip) (−) PLA_2 (Hip) (+)	pERK (Hip, DRN) (−) pERK (Hyp) (+) pAkt (Hip) (+) gK^+ (TWIK-1) (Ecx) [88]
5-HT_{1B}	Recombinant	$G_{i/o}$ (G_{i1}, G_{i2}, G_{i3}, G_o)	AC and PKA (−)		PLC, Ca^{2+} and PKC (+) nNOS (+) gK^+ (Ca^{2+} dependent) (+) gCa^{2+} (voltage dependent) (−)	pERK (Ras, PI3K) (+) pAkt (PI3K and p70 S6 kinase) (+)
	Endogenous (brain)	$G_{i/o}$ (striatum)	AC and PKA (S. nigra) (−)			pERK (+)
5-HT_{1D}	Recombinant	$G_{i/o}$ (G_{i1},G_{i2},G_{i3},G_o)	AC and PKA (−)		gK^+ (Ca^{2+} dependent) (+) gCa^{2+} (voltage dependent) (−) gCa^{2+} (N) (−)	pERK (+)
	Endogenous (brain)	$G_{i/o}$ (Hip, Cx)	AC and PKA (−)			
5-HT_{2A}	Recombinant	$G_{q/11}$	PLC, Ca^{2+} and PKC (+) PLA_2 and AA (+)	$G_{i/o}$ $G_{12,13}$	cAMP, PKC and CaM (+) PLD (Arf1) (+)	pERK (many pathways) (+) p38 kinase (RhoA) (+) pAkt (PI3K) (+) NHE (Jak2 and CaM) (+) STAT3 (Jak2) (+)
	Endogenous (brain)	$G_{q/11}$ (Cx)	PLC and Ca^{2+} (Cx, striatum) (+) PLA_2 and AA (Cx, Hip) (+)	G_i (Cx)	NOS (Cx) (+) gCa^{2+} (L) (Cx) (+) gCa^{2+} (voltage independent) (Cx, astrocytes) (+) gK^+ (delayed rectifying) (Cx) (−)	pERK (Src and β-arrestin) (Cx) (+) [89] gNa^+ (PKC) (Cx) (−)
5-HT_{2B}	Recombinant Endogenous (brain)	$G_{q/11}$ $G_{q/11}$	PLC, Ca^{2+} and PKC (+) PLC, Ca^{2+} and PKC (Cx, astrocytes) (+) PLA_2 and AA (+)	G_{13}	NOS (+) NOS (+)	pERK (Src and Ras) (+) Na^+-K^+-ATPase (PKC) (−)
5-HT_{2C} (numerous isoforms)	Recombinant	$G_{q/11}$	PLC, Ca^{2+} and PKC (+) PLA_2 and AA (+)	$G_{12/13}$ G_{i1}, G_{i3}, G_o	PLD (RhoA) (+) gK^+ (GIRK, others) (−) gCl^- (Ca^{2+} gated) (+)	pERK (many pathways) (+) pAkt (PI3K) (+) gK^+ (PKC) (−)
	Endogenous (brain)	$G_{q/11}$	PLC, Ca^{2+} and PKC (choroids plexus, Cx) (+)		NOS (choroids plexus) (+) gK^+ (delayed rectifying) (choroid plexus, striatum, Hyp) (−) [90]	pERK (Src and β-arrestin) (Cx) (+) [89] gNa^+ (PKC) (Cx) (−)
5-HT_4 (up to ten splice variants)	Recombinant	G_s	AC and PKA (+)	G_{13}	RhoA (+) Epac/Rap1 (+) gCa^{2+} (L) (+)	pERK (Src) (+) pERK (PKA and Ras) (+) NHE (Src and Ca^{2+} dependent) (−)
	Endogenous (brain)	G_s	AC and PKA (colliculus) (+)	G_{13}	RhoA (+) TTX-insensitive gNa^+ (Hip, DRN) (+) gCation (globus pallidus) (+) [91]	pERK (Src) (colliculus) (+) gK^+ (voltage and Ca^{2+} dependent) (PKA) (Hip, colliculus) (−) I_H current (Hip) (+)
5-HT_{5a}	Recombinant	$G_{i/o}$	AC and PKA (−)		PLC, Ca^{2+} and PKC (+) gK^+ (GIRK and other K^+-channel types) (+)	
5-HT_6	Recombinant Endogenous (brain)	G_s G_s	AC and PKA (+) AC and PKA (colliculus, striatum) (+)		gK^+ (striatum) (−) [90]	pERK (Fyn) (+)
5-HT_7 (4 splice variants)	Recombinant	G_s	AC and PKA (+)	G_{12}	RhoA and Cdc42 (+)	pERK (PKA and Epac) (+) pAkt (cAMP and Ca^{2+}) (+) p38 Kinase (PLCε) (+)
	Endogenous (brain)	G_s	AC and PKA (Hip) (+)	G_{12} (Hip)	RhoA and Cdc42 (Hip) (+) gK^+ (striatum) (−) [90] gCation (globus pallidus) (+) [91]	pERK (Hip) (+) I_H current (cAMP) (Hip, striatum) (+)

[a]Coupling to specific G-protein isoforms, soluble second messengers and ion channels is indicated. The table is not exhaustive. 5-HT_{1e} and 5-HT_{1F} receptors couple via $G_{i/o}$ to inhibition of AC and PKA, but almost no other information is available so they are not depicted. Observations in recombinant sites are differentiated from endogenously expressed and native cerebral receptors. Where known, the main mechanism(s) mediating the influence of 5-HT receptors on downstream signals is given. For ion channels, whether the route from receptor to current change is direct and/or via second messengers is often uncertain. For references see main text, reviews [3–5] and Refs [87–91].
[b]G-protein independent (non-G-protein-dependent pathways are in blue).[c]Abbreviations: (−), inhibition; (+), stimulation; AA, arachidonic acid; Akt, protein kinase B; CaM, calmodulin; Cx, cortex; DRN, dorsal raphe nucleus; Ecx, entorhinal cortex; Epac is a guanine nucleotide exchange factor; Fyn and Arf1 are kinase protein partners of 5-HT_6 and 5-HT_7 receptors, respectively; g, channel; Hip, hippocampus; Hyp, hypothalamus; I_{smooth}, smooth inward current; I_H, hyperpolarisation-activated current; JNK, Jun-Kinase; JAK, Janus tyrosine kinase; L, N, P/Q, types of Ca^{2+} channel; NHE, Na^+/H^+ exchanger; p, phosphorylated; Ras, Rap1, RhoA and Cdc42 are small GTPases; S. nigra, substantia nigra; Src is a tyrosine kinase; STAT, signal transducer and activator of transcription; TTX, tetrodotoxin; TWIK, tandem pore domain weakly inwardly rectifying K^+ channel.

receptors might reflect coupling to G_{i3} versus G_o, respectively [20].

By analogy, rank orders of agonist efficacy for recruiting human 5-HT_{2C} receptors differ for phospholipase (PL) C versus A_2 [21,22], and for $G_{q/11}$ versus G_{i3} [23,24]. Contrasting agonist efficacies at PLC versus PLA_2 have also been observed for human 5-HT_{2A} receptors [25], and differential coupling patterns are related to the induction of head-twitches in rats, a proxy for hallucinations in humans [22]. Thus, non-hallucinogenic agonists such as lisuride (an antiparkinsonian agent) only stimulate G_q in rat frontal cortex (FCX) [18,26], whereas hallucinogens such as psilocybin (found in magic mushrooms), mescaline (found in the peyote cactus) and lysergic acid diethylamide (LSD) stimulate both $G_{q/11}$ and $G_{i/o}$ [26]. Supporting observations of ligand-directed signaling, hallucinogenic and non-hallucinogen 5-HT_{2A} agonists also differentially influence FCX patterns of gene expression [26]. As if this was not complicated enough, hallucinogenic 5-HT_{2A} agonists elicit head-twitches independently of β-arrestin, whereas administration of 5-hydroxytryptophan (a precursor of 5-HT) induces head-twitches by harnessing a β-arrestin-dependent pathway [27]. The relationship of multiple signaling pathways to function at 5-HT_{2A} receptors is clearly complex, but these observations help explain the paradox of why some 5-HT_{2A} agonists elicit hallucinations whereas structurally related compounds do not.

It will be important to establish in more general terms whether agonist-directed trafficking is of broad physiological importance in the brain, whether its disruption is related to CNS disorders and whether it can be exploited for improving the efficacy and safety of novel therapeutic agents.

Agonist-independent constitutive activity at 5-HT receptors

Certain constitutively active GPCRs spontaneously signal (bind to G proteins) in the absence of agonists. Correspondingly, antagonists can be classified as inverse agonists (suppressing agonist-independent coupling) or neutral antagonists (doing nothing, but blocking the actions of agonists and inverse agonists), although drug actions are dependent on the stoichiometry of GPCRs to G proteins, the coupling pathway and the availability of GPCR protein partners [28,29].

Cloned human 5-HT_{1A} receptors display spontaneous coupling to $G_{i/o}$ and constitutive activity (CA) is promoted by regulator of G-protein signaling (RGS)-1 [28] (Box 1). Such protein partners can, likewise, regulate CA in the brain, where postsynaptic 5-HT_{1A} receptors in the rat hippocampus show agonist-independent coupling to G_o [30]. However, it is unknown whether 5-HT_{1A} autoreceptors likewise show CA, and the high concentration of spontaneously released 5-HT in the dorsal raphe nucleus (DRN) seems to render this notion meaningless. Moreover, although convincing evidence for constitutively active, cloned human 5-HT_{1B} and 5-HT_{1D} receptors was acquired, intensive efforts to demonstrate inverse-agonist actions of antagonists at pre and post-synaptic 5-HT_{1B} sites in the brain were unsuccessful [28].

Interestingly, non- and partially edited 5-HT_{2C} receptors display more pronounced CA towards PLC than highly edited isoforms do [25,31,32], and CA is effector-dependent, being less marked for PLA_2 [25]. Furthermore, hinting at inverse ligand-directed signaling, drugs have been described that behave as inverse agonists at PLA_2, but as neutral antagonists at PLC [25,33]. CA might be of functional significance inasmuch as 5-HT_{2C}-receptor inverse agonists more powerfully activate mesolimbic dopaminergic pathways (via GABAergic interneurons) than neutral antagonists [25,31]. Although CA is not pronounced at PLC-coupled human 5-HT_{2A} receptors, it can be amplified by overexpression of G_q and by site-directed mutagenesis of amino acids in 5-HT_{2A} receptors known to interact with G_q [25]. Interestingly, CA at cerebral 5-HT_{2A} receptors might explain impairment of associative learning by inverse agonists [25].

All splice variants of 5-HT_4 receptors display CA, which is intensified upon truncation of the C terminus [4]. Spontaneous signaling remains unreported for human 5-HT_6 receptors, although mutations in intracellular loop 3 (which interacts with G_s) render them constitutively active. Furthermore, native mouse 5-HT_6 receptors display CA for coupling to cAMP [34]. Native 5-HT_7 receptors (all variants) usually reveal CA at G_s [34], and CA was likewise seen at an alternative pathway: coupling via G_{12} to small Rho-family GTPases [35]. Several antipsychotics are inverse agonists at human 5-HT_7 receptors [34], but evidence for CA has not been obtained in the brain so functional importance remains unclear.

Fine regulation of G-protein-dependent and -independent signaling by protein partners

Proteomic strategies have revealed that 5-HT receptors interact with numerous intracellular proteins in addition to G proteins [36]. These partners control the distribution of 5-HT receptors in specific cellular domains, their trafficking in and out of the plasma membrane and signal transduction. 5-HT-receptor-associated proteins include the ubiquitous GPCR-signaling modulators, β-arrestins and others that are specific to individual 5-HT subtypes.

β-Arrestins direct the agonist-induced internalization of 5-HT_{1A} and 5-HT_4 receptors and, unprecedented for GPCRs, agonist-independent association with β-arrestins was reported for non-edited (and partially edited) 5-HT_{2C} receptors [37]. This interaction leads to constitutive internalization [37], an effect prevented by inverse agonists [32]. Intriguingly, although β-arrestins are implicated in hallucinogenic effects mediated by 5-HT_{2A} receptors (see earlier), their trafficking is independent of β-arrestin [38].

Conversely, PDZ (postsynaptic-density-95/disc-large/zonulla-occludens-1)-domain-containing proteins profoundly influence internalization of both 5-HT_{2A} and 5-HT_{2C} receptors, and PDZ proteins are essential for targeting 5-HT_{2A} receptors to dendrites in cortical neurons [39]. PDZ partners are both receptor- and function-specific. Thus, the specific sets of PDZ proteins that interact with 5-HT_{2A} versus 5-HT_{2C} receptors differ. Although PSD-95 (postsynaptic-density-95) prevents 5-HT_{2A} receptor internalization [40], it favours constitutive and agonist-dependent endocytosis of 5-HT_{2C} receptors [41]. Conversely,

MPP3 (membrane protein palmitoylated 3) stabilizes 5-HT_{2C} receptors at the plasma membrane [41] (Figure 2).

Interactions with non-PDZ proteins also influence signal transduction, as illustrated by the 5-HT_{2A} receptor. Binding of calmodulin to its C terminus impedes phosphorylation by PKC, thereby preventing desensitization [42]. Conversely, association of p90-ribosomal S6 kinase-2 with 5-HT_{2A} receptors (at their intracellular 3 loop) silences signaling [43]. In addition, a caveolin-1 interaction, established both in cerebral synapses and in glioma cells, is essential for lipid-raft targeting of 5-HT_{2A} receptors and coupling to G_q [44]. A similar role for calveolin-1 in the coupling of 5-HT_7 receptors to G_s was recently shown [45]. A further non-PDZ protein, p11, enhances the localization of postsynaptic 5-HT_{1B} receptors at the cell surface [46], and disruption of 5-HT_{1B}-receptor–p11 interactions might contribute to depressive states.

Underscoring their pleiotropic roles, protein partners also directly transduce cellular signals. For instance, β-arrestin contributes to activation of ERK by 5-HT_{2A} and 5-HT_{2C} receptors (Table 1). In addition, in colliculi neurons, 5-HT_4 receptors activate ERK independently of G_s, cAMP and β-arrestin via Src, which forms stable complexes with 5-HT_4 receptors that persist upon endocytosis [47]. 5-HT_4-receptor-mediated ERK stimulation contributes to long-term potentiation [48]. Finally, an interaction between the 5-HT_{2A} receptor C terminus and ADP ribosylation factor 1 (Arf1) is important for $G_{q/11}$-independent activation of PLD [49].

Signaling crosstalk: from functional to physical interchanges between 5-HT receptors

Scope for interactions between 5-HT receptors and other GPCRs is immense (Figures 1 and 3). Examples can be cited at all levels of integration, from input (e.g. endogenous agonist) to output (e.g. ERK phosphorylation), via G proteins themselves (recruited by several classes of 5-HT receptors). Some interchanges are facilitatory (e.g. 5-HT_4 and 5-HT_7 receptors converging via G_s onto AC), whereas others are antagonistic (e.g. 5-HT_{1A} inhibition of AC versus

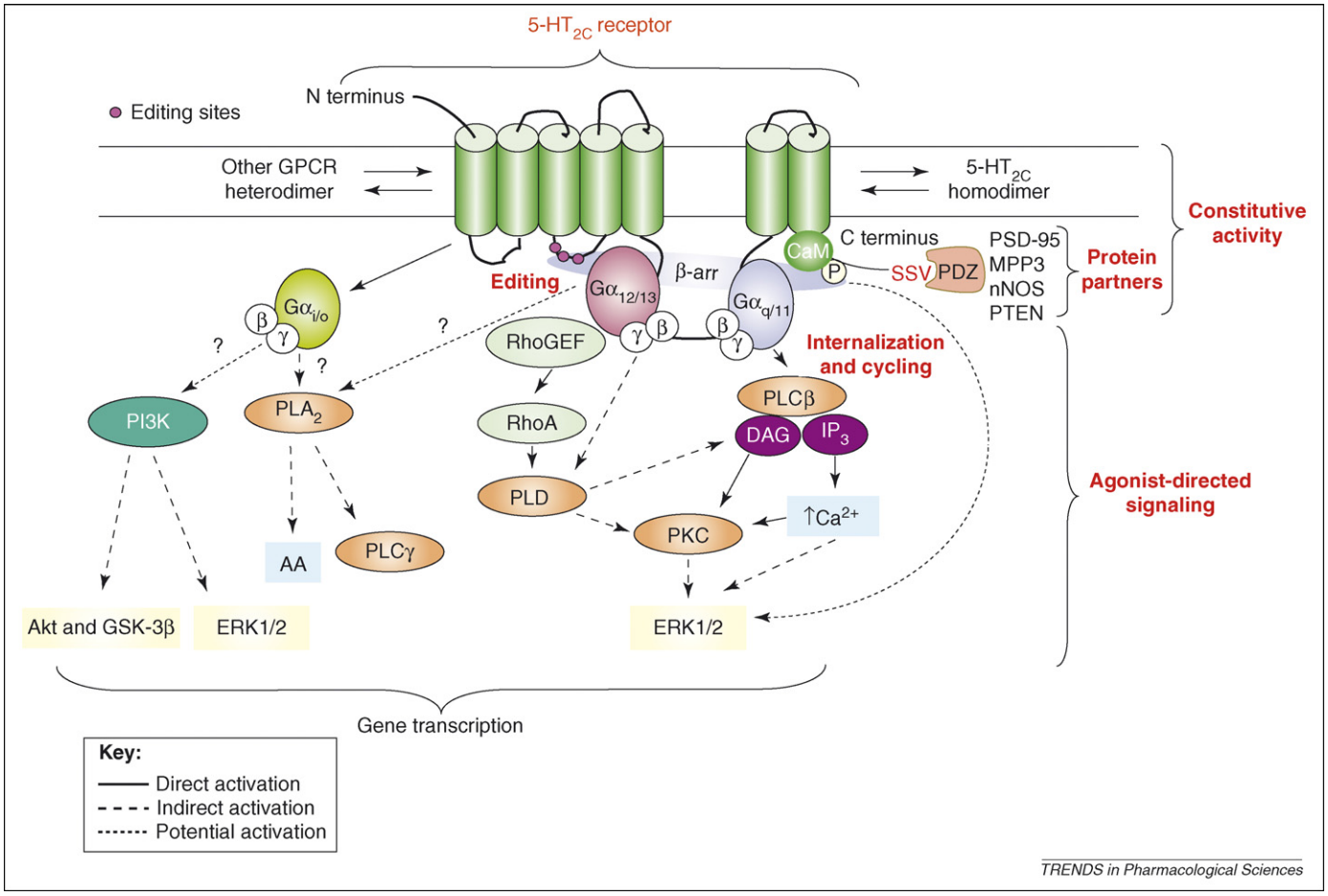

Figure 2. Signaling at 5-HT_{2C} receptors: a microcosm of cellular pharmacology. 5-HT_{2C} receptors illustrate much of the complexity of serotonin signaling. They prototypically recruit phospholipase (PL)Cβ via the α subunit of $G_{q/11}$. In addition, they possess a further important axis of transduction via PLA_2, possibly triggered by $G_{12/13}$, that also indirectly activates PLD. PLA_2 itself recruits PLCγ and cyclooxygenase 2 resulting in the generation of arachidonic acid (AA). By contrast, stimulation of PLC and PLD ultimately converges onto PKC, then results in the phosphorylation of extracellular regulated kinase (ERK)1/2. A further route to ERK activation is provided by $G\alpha_{i/o}$, which induces the phosphoinositide 3 kinase (PI3K)–Akt–glycogen synthase kinase (GSK)-3β cascade. Like ERK, this cascade controls gene transcription and it is also implicated in apoptosis and many other cellular functions. In addition to directing internalization by 5-HT_{2C} receptors, β-arrestin can activate ERK1/2. The preference of certain agonists for one transduction pathway over another is called ligand-directed signaling. Constitutive activity refers to spontaneous coupling in the absence of agonists and it is greatest for isoforms unaffected by adenosine-to-inosine mRNA editing. Although 5-HT_{2C} receptors link up with themselves to form homodimers, they probably assemble into heterodimers with other classes of GPCRs; however, this is not yet proven. Moreover, 5-HT_{2C} receptors associate with protein partners controlling membrane insertion, constitutive activity and signaling: four proteins discussed in the text are depicted, but others are known [4]. Coupling to ion channels (probably involving βγ subunits) is not shown for reasons of clarity. Abbreviations: CaM, calmodulin; DAG, diacylglycerol; il-3, intracellular loop 3; IP_3, inositol triphosphate; RhoA, Ras homolog A; RhoGEF, Ras homolog guanine nucleotide exchange factor; SSV, Ser-Ser-Val. Akt is also known as protein kinase B. '?' denotes uncertainty.

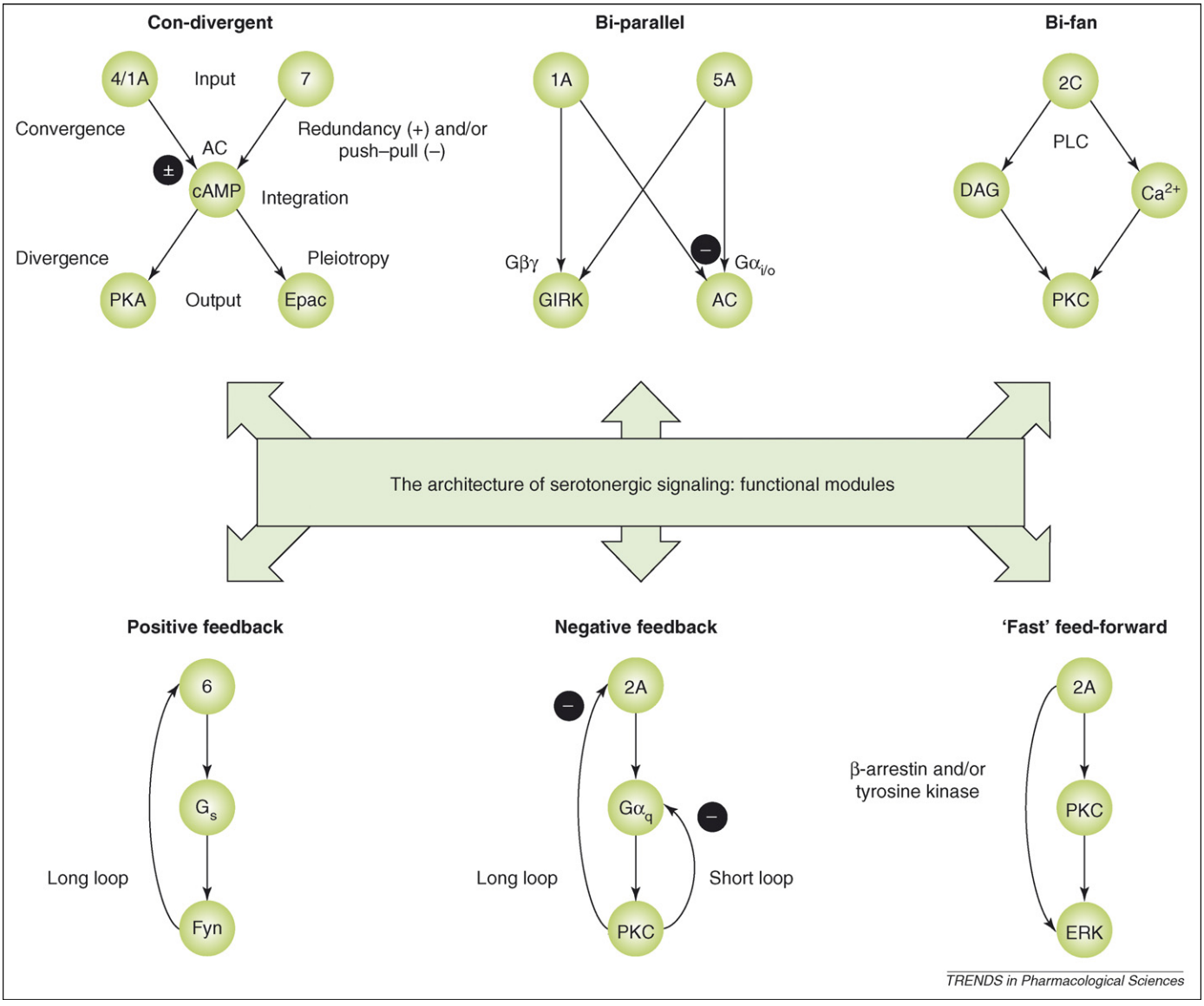

Figure 3. Cellular configurations (motifs) employed for signaling via 5-HT receptors. Signaling at 5-HT receptors is a good example of a complex cellular network and, in trying to understand its organization and operation, it is instructive to characterize specific functional units, or 'motifs' [2]. Serotonin transduction involves multiple convergent inputs onto common integrative signals, which themselves display multiple (divergent) outputs. In this fundamental 'con-divergent' module, inputs might be similar ('synergy') or opposing ('push–pull'). This is illustrated by the redundancy of 5-HT_7 and 5-HT_4 receptors, which synergistically activate adenylyl cyclase (AC), whereas 5-HT_4 and 5-HT_{1A} receptors function oppositely (push-pull) to activate and block AC, respectively. This mode of balanced control is a universal feature of complex (signaling and supra-cellular) networks; it is fundamental to homeostasis and favors resistance to disruption [2]. Similarly, the control of several pathways in a network by key links (nodes) is common; this is termed pleiotropy (multiple roles) and is exemplified by AC, which both activates PKA and recruits a further intracellular messenger, Epac. Certain other configurations used in serotonin signaling also incorporate modes of convergence and divergence (bi-parallel and bi-fan). Other motifs permit the well-known features of negative feedback, positive feedback or 'fast' feed-forward. All inputs are positive unless indicated. The number indicated in the circle corresponds to the subtype of 5-HT receptor. Abbreviations: DAG, diacylglycerol; TK, tyrosine kinase.

5-HT_4 stimulation). Although most exchanges are functional, recent work has revealed physical interactions. Several topical examples are outlined here.

The varying fates of tryptophan (Figure 1) emphasize crosstalk at the biosynthetic stage, and endogenous ligands represent a further surprising element of communication. Thus, tryptamine is a weak agonist of 5-HT receptors, its actions potently mimicked by recently discovered thyronamine trace amines [2]. In addition, dopamine engages 5-HT_{2A} receptors and triggers their internalization [50].

Numerous cases of reciprocal phosphorylation-mediated crosstalk between 5-HT receptors and other classes of GPCR occur; these are usually mediated via PKA and PKC, which thereby modulate plasma-membrane expression, coupling and interactions with protein partners [51,52]. In addition, common protein partners of two 5-HT receptors represent a potential mode of crosstalk. This might be involved in the reciprocal influence of co-expressed 5-HT_{1B} and 5-HT_{2B} receptors on their contrasting patterns of clathrin- or caveolin-controlled internalization [53].

Like other GPCRs, 5-HT receptors transactivate tyrosine-kinase receptors. For example, one pathway of 5-HT_{2A}-receptor-mediated ERK phosphorylation is dependent on Src, calmodulin and transactivation of epidermal-growth-factor (in neuronal cells) or fibroblast-growth-factor receptors (in glial cells) [54,55]. In addition, trans-

activation of tyrosine-kinase receptors enhances the expression of neurotrophic factors such as brain-derived neurotrophic factor; this action might be involved in 5-HT-induced cell survival, proliferation and neurogenesis [2,5].

Oligomeric associations of GPCRs often comprise signaling units, and 5-HT_{1A}, 5-HT_{1B}, 5-HT_{1D}, 5-HT_4 and 5-HT_{2C} receptors all form dimers [56,57]. In contrast to some classes of GPCRs, agonist binding does not greatly influence formation of 5-HT-receptor dimers, indicating constitutive assembly before membrane insertion. Underpinning this contention, 5-HT_{2C} receptors generate dimers in the endoplasmic reticulum and Golgi of living cells [58]. 5-HT_{2C} dimers possess an interface between transmembrane helices IV and V, and dimer proximity is increased and decreased by agonists and inverse agonists, respectively [59]. Furthermore, analysis of functionally compensating, co-expressed mutant 5-HT_{2C} receptors linked to G_q (fusion proteins) indicates that the dimer is asymmetric versus G_q, with both subunits binding to 5-HT and having distinct roles during signaling [58,59]. 5-HT_{2C}-receptor dimers comprise a conformational heterodimer, supporting the hunch [31] that they might, by analogy to 5-HT_{1B} and 5-HT $_{1D}$ heterodimers [57], associate with other GPCRs. Indeed, 5-HT_{2A} and metabotropic glutamate 2 ($mGlu_2$) receptors assemble into heterodimers via a transmembrane-4 and -5 linking domain [60]. Intriguingly, $mGlu_2$ receptor agonists blunt heterodimer coupling to G_i providing one substrate for their anti-hallucinogenic properties, although reduction of glutamate release from thalamocortical afferents might also be involved [60,61].

Direct evidence has been acquired for $mGlu_2$–5-HT_{2A} heterodimers in the human brain and for a reduced density in schizophrenics [60]. Nonetheless, this remains one of the few demonstrations of heterodimers in neuronal tissue. Future studies must focus on the putative heterodimerization of native 5-HT receptors *in situ* and on their pharmacological profiles in the hope of identifying novel targets for therapeutic intervention.

Future research directions

Concretizing recent developments

One important avenue for future research will be the confirmation of these recent developments in serotonin signaling, their further characterization in the brain and clarification of their functional and therapeutic significance. The following topics are also likely to attract particular interest over the coming years.

Signaling at 5-HT receptors in the brain: return to the future

It is important to distinguish the plethora of data from solitary recombinant 5-HT receptors expressed in cell lines from rare observations on native brain populations in their 'natural' environment. Alhough harder to acquire, the latter are decisive in understanding real-life signaling pathways. Correspondingly, there is increasing interest in serotonin signaling where this all started, in the brain itself, albeit with the benefits of modern technologies. For example, studies showing that long-term antidepressant treatment desensitizes signaling at 5-HT_{2A} and (presynaptic) 5-HT_{1A} receptors can be amplified by autoradiographic analyses of G-protein coupling and antibody-based studies of individual G-protein isoforms [2,51]. More work on the influence of drugs and stimuli such as stress on 5-HT-receptor coupling is needed, in particular in view of regional differences. For example, various populations of 5-HT_{1A} receptors differentially couple to distinct G-protein isoforms with hippocampal and raphe sites privileging G_o (G_z) versus G_{i3}, respectively [20,51,62]. Moreover, pre- versus post-synaptic sites mainly signal via GIRK versus AC (Figure 1), and 5-HT_{1A} receptors in the hippocampus and hypothalamus are facilitatory and inhibitory to ERK phosphorylation, respectively [63]. Complementing studies of brain tissue, studies of primary neuronal cultures and cells bearing endogenously expressed receptors can also yield important information. For example, CA was recently demonstrated at 5-HT_{2C} receptors in cultured cortical neurons [32].

Finally, although human brain tissue is seldom available, signaling in humans can be monitored using peripheral markers, including platelets bearing SERTs and 5-HT_{2A} receptors. Furthermore, leukocytes express tryphophan hydroxylase, SERTs, 5-HT_3 receptors and several classes of G-protein-coupled 5-HT receptors displaying coupling patterns similar (although not identical) to those found in the brain [64–66].

Signaling in non-neuronal cells

Cytokine-releasing lymphocytes derived from the systemic circulation access the brain where they unite with resident microglia, the macrophages of the brain. Upon activation, microglia influence neuronal function and serotonin transmission by releasing cytokines, NO, glutamate and reactive oxygen species. Immune-competent cells are implicated in processes related to neurodegeneration, depression and schizophrenia. Microglial 5-HT_7 receptors couple via G_s to the generation of interleukin-6 and, extrapolating from peripheral macrophages, microglia probably possess other 5-HT-receptor subtypes [67]. 5-HT_7 receptors are also expressed by T-lymphocytes, which they activate via an ERK-dependent pathway. Lymphocyte-localized 5-HT_{1A} receptors, likewise, stimulate ERK, and 5-HT_{1B} receptors provoke T-cell proliferation [64].

Oligodendrocytes, which surround the axons of neurons, generate myelin, provide trophic support and modulate neurotransmission. Their dysregulation is implicated in schizophrenia and depression [68]. Supporting interest in serotonergic signaling at oligodendrocytes, their peripheral counterparts, Schwann cells, bear 5-HT_{2A} receptors activated by 5-HT released from endoneurial mast cells and coupled to Ca^{2+} flux [69].

In addition to structural, energetic and 'nursing' roles, astrocytes modulate neurons, microglia and blood vessels by release of various mediators. Despite the long-established presence of mRNA for SERT and multiple 5-HT receptors (G-protein coupled and 5-HT_3) in astrocytes [70], little is known of signaling. Nonetheless, what we do know is intriguing. Engagement of 5-HT_7 receptors in astrocytes triggers interleukin-6 synthesis by a p38- and PKCε-dependent pathway [71]. Curiously, 5-HT_7 coupling to AC is enhanced upon chronic exposure to antidepressants [2], and astrocytic 5-HT_{2B} receptors, which control intracellu-

lar Ca^{2+}, are also upregulated by fluoxetine [72,73]. 5-HT_{2A} receptors, likewise, couple to Ca^{2+} and PLC [72] and their recruitment transactivates fibroblast-growth-factor receptors to liberate glial-cell-derived neurotrophic factor, which is involved in neurogenesis [55]. Moreover, activation of 5-HT_{1A} receptors in astrocytes releases a neurite-extending growth factor, S-100b, independently of AC [74].

Interest in non-neuronal signaling is not confined to psychiatry. Modulation of GPCR-directed signals in lymphomas and gliomas, the major form of cerebral tumor, is attracting attention. Combined actions at 5-HT receptors and downstream kinases could lead to improved anticancer agents for the brain [2,66]. Finally, the polyomavirus JC virus (JCV) provokes multifocal leukoencephalopathy in immuno-compromised patients after penetration of astrocytes and oligodendrocytes via 5-HT_{2A} receptor-mediated, clathrin-dependent endocytosis and ERK activation; 5-HT_{2A} antagonists abrogate infection [75].

Signaling into function

A major challenge is to integrate serotonergic signaling with CNS function and disease. Knowing how a 5-HT receptor affects signaling in a specific class of cell is a step in this direction, hence the importance of characterizing transduction in defined neurons, like glutamatergic pyramidal cells in FCX and ventrotegmental dopaminergic neurons. Furthermore, pursuing cascades from 5-HT receptors through to functionally relevant outputs such as neuronal firing rates and transcription factors is instructive. However, it is necessary to directly illuminate the black box between coupling and behavior. For example, G_q-mediated signaling at 5-HT_{2A} receptors in FCX might be related to schizophrenia, an interpretation corroborated by a complementary strategy showing that hallucinogen-induced head twitches are abolished in mice lacking 5-HT_{2A} receptors [76]. A similar genetic approach has revealed a role for 5-HT_{1A}-receptor coupling to G_z in the modulation of endocrine function [51,62].

Signaling into therapeutics

The ultimate goal is to translate improved knowledge of serotonergic signaling into therapeutics. Two complementary strategies envisage interventions: (i) at 5-HT receptors, and (ii) (more speculatively) downstream at signaling pathways. Conceptually speaking, multiple targets invite multi-target drugs, so network-based approaches are better adapted to modulation of serotonergic signaling than highly selective agents [2]. Accordingly, most targets are best modulated in combination with others.

For example, optimal recruitment of the AC–PKA–Epac axis could be achieved by mixed agonists at 5-HT_4 and 5-HT_7 receptors. In addition, stimulation of ERK1/2 and Akt by 5-HT_{2A} and 5-HT_7 receptors exploits complementary Ca^{2+}- and cAMP-dependent mechanisms, and Epac is implicated in the enhancement by 5-HT_7 receptors of the ERK response to 5-HT_{2A} sites [77]. Although the therapeutic vocation of mixed 5-HT_{2A} and 5-HT_7 agonists is unclear, concomitant stimulation of 5-HT_{1A} receptors and blockade of 5-HT_{2C} receptors is a core effect of atypical antipsychotics such as ziprasidone [1]. Of particular interest are ligands specific for 5-HT_{2A}–$mGlu_2$ complexes, or other putative heterodimers possessing distinctive binding and coupling profiles [60]. In regard to CA, antipsychotics behaving as 5-HT_{2A} inverse agonists might have efficacy against negative symptoms without evoking motor perturbation [1]. In addition, 5-HT_{2C} inverse agonists could be more powerful antidepressants than neutral antagonists; however, their discontinuation is more likely to provoke withdrawal [31]. Finally, with a view to exploiting ligand-directed signaling, 5-HT_{2A}-receptor agonists favoring G_q versus G_i activation and devoid of hallucinogenic properties are an attractive possibility.

Intracellular signals are inherently challenging targets because they are often ubiquitous; indeed, drug vectorization comes from GPCRs, not transduction pathways! Nonetheless, multi-target drugs acting at two key nodes in a signaling network offer one answer. For example, mixed 5-HT_4 agonists and phosphodiesterase-4 inhibitors should primarily reinforce cAMP transduction in regions where 5-HT_4 receptors are activated, improving efficacy and safety of such pro-cognitive agents [2]. A further concept is manipulation of interactions between 5-HT receptors and protein partners. One concrete example is the use of small peptides to decouple 5-HT_{2C} receptors from their PDZ partners, mimicking the desensitization elicited by antidepressants [41]. Conversely, blocking interactions between 5-HT_{2C} receptors and the phosphatase PTEN (phosphatase with tensin homology) reproduces the inhibition by 5-HT_{2C} agonists of the excitation of mesolimbic dopaminergic neurons by cannabinoids, preventing their rewarding effects [78]. Hence, interference with the association between 5-HT_{2C} receptors and PTEN might counter drug addiction. Another possibility is simultaneous actions at 5-HT receptors and RGS-4, a susceptibility gene for schizophrenia that interacts with 5-HT_{1A} receptors [1,79]. Intriguingly, cellular prion protein modulates signaling crosstalk among 5-HT_{1B}, 5-HT_{2A} and 5-HT_{2B} receptors, and its pathological counterpart, scrapie prion protein, impacts serotonergic neurons. This indicates that serotonergic transduction might be a target for control of spongiform encephalopathies [80]. Finally, underscoring prospects that drugs can directly affect intracellular signals, agents blocking the interaction between 5-HT_6 receptors and G_s were recently unveiled [81].

Concluding remarks

Signaling via G-protein-coupled 5-HT receptors is extraordinarily diverse, and the recent developments discussed here underline its complexity. There is an urgent need to further our understanding of serotonergic transduction in discrete cerebral regions, in defined classes of neurons, in response to therapy and under physiological and pathological conditions. Also, as accentuated here, signaling in non-neuronal cells should not be neglected. Finally, the functional significance of specific serotonergic signaling pathways awaits clarification. Improved knowledge of how 5-HT exerts its actions at the cellular level should ultimately permit therapeutic exploitation of innovative concepts like CA, heterodimers and agonist-directed signaling that, despite oceans of speculative ink, remain of unproven clinical relevance.

Much has been achieved, but still more remains to be learned if knowledge of serotonin signaling is to be trans-

lated into tangible benefits for those with disorders related to its perturbation.

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