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# 5-HT$_6$ Receptor: A New Player Controlling the Development of Neural Circuits

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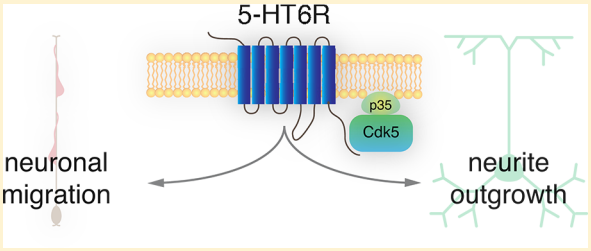
**ABSTRACT:** 5-HT$_6$ receptor (5-HT6R) is a G protein-coupled receptor that has recently emerged as a new regulator of neural development. In addition to the canonical Gs adenylyl cyclase pathway, recent proteomics approaches reveal that 5-HT6R is able to engage key developmental signaling pathways controlling neuronal circuit formation, neuronal connectivity, and psychiatric-relevant behaviors. For example, at early stages of neuronal development, expression of 5-HT6R constitutively regulates the activity of the cyclin-dependent kinase (Cdk)5 and, through this mechanism, controls cellular processes involved in circuit formation, including neuronal migration and neurite outgrowth. In addition to the Cdk5 pathway, 5-HT6R modulates a variety of key developmental targets such as Fyn, Jab1, and mammalian target of rapamycin (mTOR). Engagement of developmental pathways through 5-HT6R pharmacological manipulation has led to interesting new therapeutic perspectives in the field of psychiatric-related disorders. Indeed, 5-HT6R blockade can rescue a pathological overactivation of the mTOR pathway induced by early life insults in rodents and normalizes the associated social and episodic memory deficits. Here, we review recent evidence supporting the notion that 5-HT6R is at the interface of key developmental signaling pathways and a novel actor in the orchestration of neural circuit formation.




**KEYWORDS:** *5-HT6R, CDK5, development, migration, cortex*

Serotonin acts as an important developmental signal that regulates a wide variety of cellular processes involved in the assembly of neural networks.[1] In rodents and primates, early life serotonin deregulation has been associated with the emergence of complex psychiatric phenotypes affecting stress physiology, emotions, aggression, and social behaviors.[2,3] Serotonin has been detected in the brain of embryos at early stages of development, and many different types of serotonin receptors are expressed during brain development.[4−6] The precise cell-type-specific expression of serotonin receptors during development and their function in regulating cellular processes involved in neural circuit formation are starting to be characterized.[7] Among potential targets of early life serotonin, 5-HT$_6$ receptor (5-HT6R) has recently emerged as an interesting new player. The diversity of developmental processes and cell types controlled by 5-HT6R during neural circuit formation remains to be fully characterized. Emerging data indicates that 5-HT6R plays a key role in mediating developmental defects induced by an excess of serotonin during the early process of neurulation.[8] More recently, cell-type genetic manipulation of 5-HT6R during later steps of corticogenesis has provided evidence that 5-HT6R plays an important role in regulating neuronal migration.[9] In addition, *in vitro* findings have revealed that 5-HT6R promotes neurite outgrowth in a cell-autonomous manner.[10] Further supporting a critical role of 5-HT6Rs, studies have revealed that 5-HT6R binds to a variety of signaling proteins known to control neurodevelopmental processes such a neuronal migration, neurite growth, and dendritic spine morphogenesis. Finally, behavioral studies have demonstrated that 5-HT6R can functionally recruit some of these developmental pathways to modulate the emergence of schizophrenia-related cognitive deficits induced by early life insults.[11] In this review, we aim to present these new findings and defend the idea that 5-HT6R is a novel key actor regulating neural circuit assembly.

## 5-HT6R CONTROLS KEY DEVELOPMENTAL SIGNALING PATHWAYS

5-HT6R is a Gs-coupled receptor that activates cAMP formation upon agonist stimulation in numerous recombinant systems[12,13] as well as in native systems such as primary cultured striatal neurons[14] or pig caudate membranes.[15] Notably, 5-HT6R shows a substantial level of constitutive activity at Gs signaling both in cell lines[9,16] and primary cultured striatal neurons endogenously expressing 5-HT6R (S.C.-D. and P.M., unpublished results), suggesting that it is

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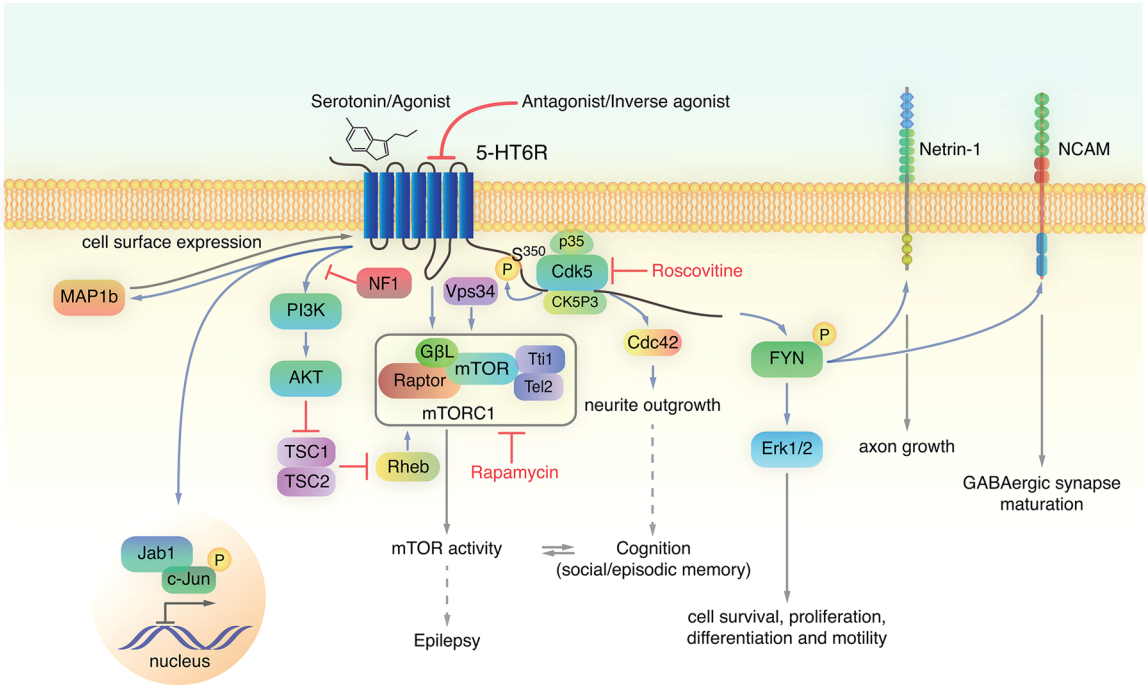


**Figure 1.** Neurodevelopmental signaling pathways engaged by 5-HT6Rs. Signaling pathways involved in neurodevelopmental and cognitive processes controlled by 5-HT6Rs and 5-HT6R-interacting proteins controlling its functional activity discovered from two-hybrid screens and AP-MS proteomic strategies are illustrated. Note that Fyn not only is an intermediate signal contributing to 5-HT6R-mediated Erk1/2 activation but also that it promotes receptor cell surface expression. Likewise, Jab1 increases receptor localization at the plasma membrane.

able to transduce signals even in the absence of serotonin. However, it became evident that 5-HT6R coupling to Gs does not account for its effects upon neuronal migration and differentiation, raising the possibility that alternative coupling mechanisms might be involved: control of interneuron (IN) migration by 5-HT6 agonists was only partially affected by inhibitors of the cAMP pathway,[17] and migration defects observed after 5-HT6R downregulation induced by *in utero* electroporation of a 5-HT6R shRNA were rescued by the re-expression of a 5-HT6R mutant not able to couple to Gs (Gs-dead mutant) in the absence or presence of an agonist.[9] Moreover, expression of the Gs-dead 5-HT6R mutant promoted neurite outgrowth of neuroblastoma cells and primary neurons similar to that of the wild-type receptor.[10] Likewise, it is unlikely that the inhibition of 5-HT6R-elicited cAMP production by antagonists would underlie their procognitive action observed in a wide range of models of cognitive impairment in rodents, as the cAMP pathway generally has a favorable influence upon cognition.[18]

This provided the impetus for two-hybrid and proteomics screens aimed at identifying 5-HT6R-associated partners, in line with increasing evidence indicating that G-protein-coupled receptors (GPCRs) can interact with large protein complexes that finely modulate their functional activity, including proteins directly involved in signal transduction. The Src family tyrosine kinase Fyn was the first 5-HT6R interacting protein identified by means of a two-hybrid screen using the carboxy-terminal domain of the receptor as bait.[19] Further studies showed that the physical association of 5-HT6R with Fyn promotes receptor cell surface expression and its coupling to G-proteins and, reciprocally, that 5-HT6R activation increases Fyn kinase activity, as assessed by the increase in Fyn $Tyr^{420}$ phosphorylation measured upon 5-HT6R stimulation by serotonin.[19] Moreover, data show that activation of the extracellular-regulated kinase (Erk)1/2 pathway by 5-HT6R is dependent on Fyn[19] (Figure 1). Fyn is known to regulate the survival, proliferation, differentiation, and motility of many cell types, and several lines of evidence suggest that it is a critical player in brain development. For instance, the combined deletion of Fyn and Src in mice induces a reeler-like phenotype, i.e., the inversion of the cortical plate and defects in cerebellar Purkinje cell migration, whereas brains from embryos deleted of Src seem to be normal and the sole deletion of Fyn induces an intermediate phenotype.[20] This suggests a role of Fyn (in combination with Src) in the reelin signaling pathway controlling cortical lamination and the formation of the Purkinje cell plate. Beyond migration, Fyn has also been involved in netrin-1-mediated axon growth of cortical neurons[21] and in the maturation of GABAergic synapses elicited by the neural cell adhesion molecule (NCAM).[22] Whether Fyn contributes to the control of cortical neuron migration and neurite growth by 5-HT6Rs remains to be established.

Two-hybrid experiments performed by the same group of investigators identified two additional 5-HT6R partners, namely, Jun activation domain-binding protein 1 (Jab 1)[23] and the microtubule-associated protein Map1b.[24] 5-HT6R stimulation promotes Jab1 translocation to the nucleus, its association with c-Jun, and c-Jun phosphorylation[23] (Figure 1). Moreover, concomitant upregulation of 5-HT6R and Jab1 was found in focal cerebral ischemia induced by middle cerebral artery occlusion, and it has been suggested that Jab1 may act as a cell protector against ischemic/hypoxic insults.[23] Like Jab1, Map1b increases 5-HT6R cell surface expression and consequently receptor-mediated signal transduction[24] (Figure 1). Again, the influence of the interaction between 5-HT6R and Jab1 or Map1b in its control of neurodevelopmental processes remains to be established.



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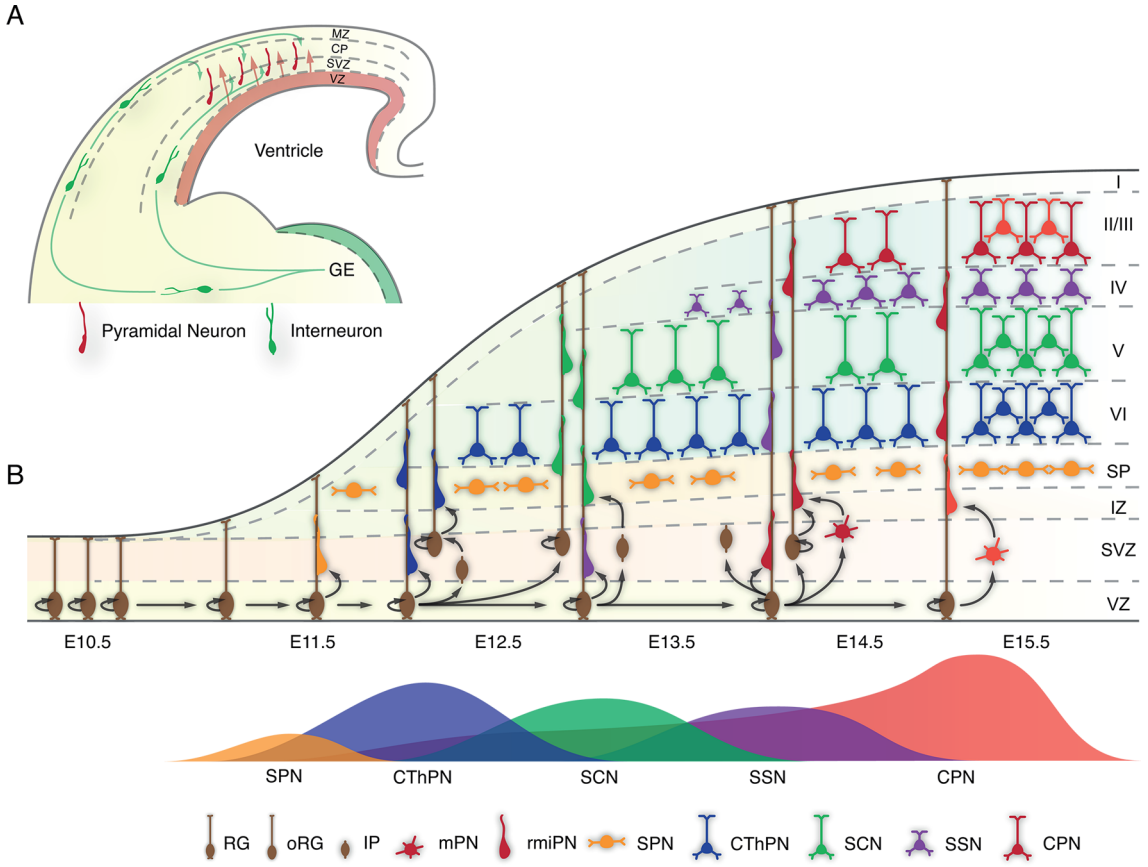


**Figure 2.** Cellular processes involved in cortical circuit assembly. (A) Excitatory projection neurons (PNs, red) are generated in the ventricular zone (VZ) of the dorsal pallium, whereas inhibitory cortical interneurons (INs, green) are produced in the ganglionic eminence (GE). (B) Cortical layers are built following an inside−outside temporal sequence. Radial glial (RG) and intermediate progenitors (IP) increase the pools of newborn neurons, which transit from a multipolar state (mPN) to radial migration (rmPN). Following the generation of subplate neurons (SPN), early born PNs are generated between E11.5 and E13.5 and give rise to layer VI cortico-thalamic neurons (CthPN) and layer V subcortical projection neurons (SCN). Late-born PNs are generated mainly between E14.5 and E16.5 and give rise to layer IV spiny stellate neurons (SSN) and layer II/III callosal projection neurons (CPNs). The 5-HT6R/Cdk5 complex controls the migration of late-born PNs.

Proteomics screens combining affinity purification of receptor interacting proteins and their identification by mass spectrometry (AP-MS) identified, in parallel, several dozen candidate partners of 5-HT6R.[10,11] Notably, 5-HT6R's interactome showed an enrichment in proteins known be involved in brain development, learning, and synaptic plasticity, consistent with the role of the receptor in neurodevelopmental processes and cognition. [11] These include several proteins of the mammalian target of rapamycin (mTOR) pathway, such as mTOR itself and raptor, which, together with mTOR, constitutes the mTOR complex 1 (mTORC1) sensitive to rapamycin[25−27] (Figure 1). In contrast, no proteins specific for mTOR complex 2 (mTORC2) were identified, suggesting that 5-HT6Rs selectively interact with mTORC1. Proteins identified as 5-HT6R-associated partners also include Tti1 and Tel2, which form a complex required for the assembly and stability of mTORC1, [28] and the small GTPase Rheb (Ras homologue enriched in brain), which directly activates mTOR[27] (Figure 1). In addition, 5-HT6R recruits neurofibromin1 (NF1), a Ras GTPase activating protein identified as an upstream modulator of the mTOR pathway, [29] and Vps34, a class III phosphatidylinositol 3-kinase (PI3K) necessary for mTORC1 activation in response to amino acids and implicated in autophagosome formation[30] (Figure 1).

The mTOR pathway plays a critical role in various neurodevelopmental mechanisms ranging from neurogenesis, neuron migration, and dendritic development to dendritic spine morphogenesis.[31] Consequently, deregulation of mTOR has been involved in a number of neurodevelopmental disorders such as tuberous sclerosis,[32−34] Fragile X,[35,36] and Rett syndromes,[37] which are often characterized by migratory defects, neuron mispositioning, and morphological abnormalities and result in a wide range of symptoms including intractable epilepsy, cognitive deficits, autism symptoms, and mental retardation. The remarkable enrichment in 5-HT6R's interactome of proteins in the mTOR pathway and their implication in several key steps of brain development prompted further studies, which showed that the stimulation of 5-HT6R elicited by peripheral administration (or local delivery) of specific agonists induces mTOR activation in various brain regions including the striatum (the structure expressing the highest receptor density) and the prefrontal cortex (PFC).[11] Further supporting the ability of 5-HT6Rs to engage the mTOR pathway, a recent study showed a concomitant increase in 5-HT6R expression and mTOR activation in the hippocampus of rats treated with pilocarpine, a model of temporal lobe epilepsy, and mTOR activation was strongly reduced by a pretreatment with a 5-HT6 antagonist (SB399885) for 3 days prior to pilocarpine administration. Moreover, the 5-HT6


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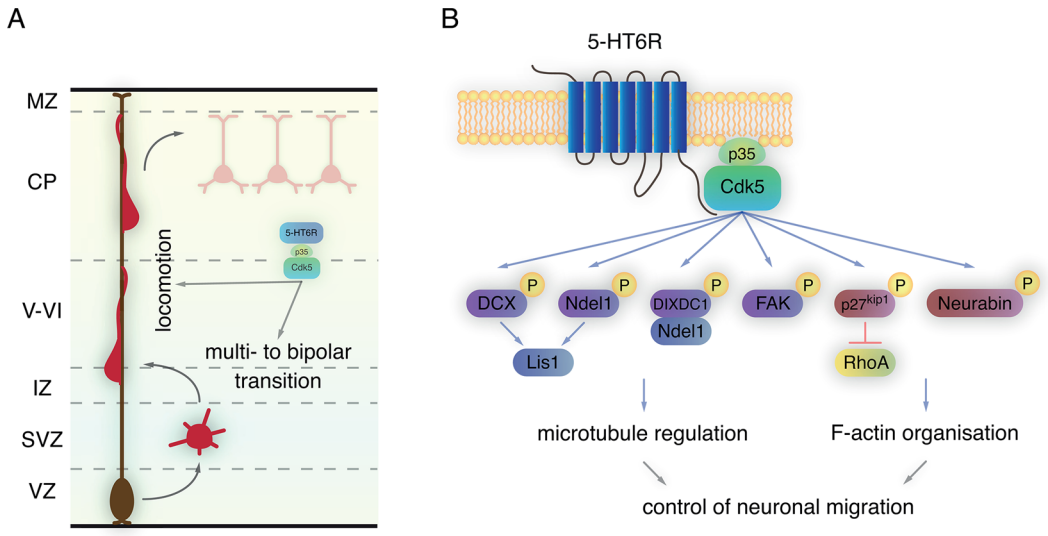


**Figure 3.** 5-HT6R/Cdk5 pathway controls pyramidal neuron migration. (A) The 5-HT6R/Cdk5 complex controls two distinct steps in the migratory process of pyramidal neurons: the polarity switch from a multipolar to a bipolar state and the phase of glial-guided locomotion. (B) The 5-HT6R/Cdk5 complex phosphorylates focal adhesion kinase (FAK) and the microtubule-associated protein doublecortin (DCX). Several other important Cdk5 downstream targets control cytoskeleton dynamics and neuronal migration, including the lissencephaly 1 binding protein Ndel1, disheveled-axin domain containing-1 (Dixdc1), the Cdk inhibitor protein $p27^{kip}$, and neurabin.

antagonist increased latency of seizures and reduced their severity, suggesting a role for the 5-HT6R/mTOR pathway in epileptogenesis.[38] Experiments performed in cell cultures showed that mTOR activation by 5-HT6Rs depends on both the physical interaction of mTOR with the receptor's C-terminal domain and the canonical PI3K/Akt pathway classically involved in mTOR activation by growth factor receptors.[11]

In addition to proteins of the mTOR pathway, proteomics screens revealed the interaction of 5-HT6Rs with a network of proteins including cyclin-dependent kinase (Cdk)5, some of its regulators, and some of its substrates known to control actin cytoskeleton dynamics and to be involved in neurodevelopmental processes such as neuronal migration, neurite growth, and synapse morphogenesis[9,10,39,40] (Figure 1). Notably, 5-HT6R/Cdk5 association occurs in the absence of agonist and is not further enhanced by agonist-induced receptor stimulation but can be disrupted by antagonists, indicating that this interaction is a dynamic process dependent on the receptor's conformational state. In line with these findings, 5-HT6Rs were found to induce activation of Cdk5 in an agonist-independent manner both in a neuroblastoma-glioma cell line and primary embryonic neurons expressing native receptors.[10] Moreover, receptor-mediated Cdk5 signaling required 5-HT6R phosphorylation at $Ser^{350}$ by associated Cdk5 (Figure 1). These findings suggest a reciprocal modulation of 5-HT6R and Cdk5 functional activities mediated by the association of both partner proteins and reveal a novel mechanism underlying agonist-independent activation of GPCRs, which depends on receptor phosphorylation by an associated protein kinase.[10,41]

Collectively, these interactomics studies highlight the ability of 5-HT6R to engage key developmental signaling pathways potentially involved in its control of neuronal circuit formation and neuronal connectivity, in addition to the canonical Gs adenylyl cyclase pathway.

## CORTICAL CIRCUIT FORMATION: A COORDINATED MULTICELLULAR AND MULTISTEP PROCESS

Recent evidence indicates that 5-HT6R regulates different types of cellular events involved in cortical circuit formation. In mammals, the cerebral neocortex has emerged as a six-layered laminar structure composed of about 80% excitatory glutamatergic pyramidal neurons (PNs) and 20% inhibitory GABAergic interneurons (INs).[42,43] Although many morphological, functional, and cellular properties distinguish the rodent cortex from the primate cortex,[44] it is thought that a basic cortical microcircuit template is conserved in rodents and humans and that its massive expansion in humans is due to its evolutionary success.[45] Much progress has been made in recent years to better define the molecular, morphological, and functional properties of different cortical neuron subtypes, but the diversity of PN and IN subtypes is much larger than anticipated.[45−47] Developmental studies have provided a basic set of principles that determine the emergence of different cortical neuron subtypes and their integration into a functional microcircuit.

Early steps of forebrain development involve neural tube formation and the initiation of transcriptional programs that determine the identity of dorsal pallial and ventral subpallial progenitor domains.[48] These processes take place between E7.5 and E10.5 in mice and precede the phase of cortical neurogenesis.[48] In contrast to INs, which arise in the ganglionic eminences of the subpallium, PNs are generated in the ventricular zone of the dorsal pallium (Figure 2A).[42,49] The identity and laminar positioning of PNs are largely determined by their date of birth. In mice, early born PNs are generated from the asymmetric division of radial glial cells from E11.5 to E13.5 and give rise to deep cortical layers (layers V and VI), whereas late-born PNs are generated from E14.5 to E16.5 and differentiate into upper-layer PNs (layers II−IV)[42,43,50] (Figure 2B). In order to assemble into cortical circuits, PNs and INs first migrate to reach their final laminar position in the developing cortex. INs exit the subpallium through a process of


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tangential migration before invading the developing cortex, whereas PNs migrate radially from the dorsal ventricular zone[49,51,52] (Figure 2A). Distinct steps in the migration of late-born PNs have been identified. First, late-born PNs navigate into the subventricular zone (SVZ) and intermediate zone (IZ) of the developing cortex, where they remain in a multipolar state, characterized by the dynamic extension and retraction of neurites[53−55] (Figure 3A). During this phase of migration, PNs migrate over short distances and tangentially disperse into adjacent cortical columns.[54,55] Following this transition period, PNs switch to a polarized bipolar morphology and attach to radial glia (RG). The transition from a multi- to a bipolar morphology is a vulnerable period, and alterations in this finely regulated process lead to the ectopic accumulation of PNs in the white matter.[43,51,56] Following this switch, PNs use glia-guided locomotion to ascend radially toward the cortical plate and then detach from RG fibers and reach their final laminar position through a final step of somal translocation.[51] Once established in their correct laminar position, they develop dendritic arbors and integrate into neural networks by forming functional synapses (Figure 3A). The rules that govern cortical cell-type-specific wiring are still largely unknown but involve many different types of guidance cues and protein complexes that cooperate in synapse formation at the pre- and postsynaptic levels.[57,58]

## ROLE OF 5-HT6R IN THE EARLY STEPS OF BRAIN DEVELOPMENT

Using high-performance liquid chromatography to measure serotonin levels in the fetal brain, it was shown that serotonin is present as early as E10.5 in the developing forebrain and that the placenta represents the main source of serotonin for the forebrain until E15.5.[4] By E16.5, axonal projections from the raphe nuclei have reached the forebrain and become the main provider of serotonin during the late phase of embryonic cortical circuit assembly.[59] Sources of serotonin to the brain before E10.5 remain to be fully established. Interestingly, monoamine oxidase isoform A (MAO-A), the main enzyme controlling the degradation of serotonin, is expressed during the process of neurulation (E7.5−E10.5).[60] SiRNA-mediated knockdown of MAO-A in whole embryonic cultures from E7.5 to E10.5 leads to excessive amounts of serotonin in embryos and to severe brain growth defects.[60] Microinjections in the amniotic cavity of an excess of serotonin mimics the brain retardation phenotype caused by MAO-A knockdown. In addition, pharmacological inhibition of serotonin biosynthesis by *para*-chlorophenylalanine (PCPA) microinjections fully rescues both the excess of serotonin as well as the developmental defects induced by MAO-A knockdown. Taken together, these data strongly support the notion that an excess of serotonin is the major effector underlying neurulation defects induced by MAO-A loss-of-function. Investigation of the downstream targets of high serotonin revealed that 5-HT6R is the key receptor contributing to the developmental consequences of MAO-A knockdown during neurulation. Indeed, 5-HT6R knockdown fully rescued the severe brain alterations caused by MAO-A knockdown or microinjections of exogenous serotonin.[60] The cellular processes controlled by 5-HT6R during neurulation remain to be fully characterized but are likely to involve a decrease in apoptosis and proliferation of neuroepithelial cells.[8,60] Whether these progenitors express 5-HT6R during neurulation and whether serotonin can directly activate developmental pathways in these cells remain to be established.

## ROLE OF 5-HT6R IN CORTICAL NEURON MIGRATION

Serotonin increases during the late phase of embryonic cortical development and originates from raphe fibers invading the subplate and marginal zone.[4] The late phase of embryonic cortical circuit assembly is characterized by intense neuronal migration of late-born PNs and INs[49,52] (Figure 2). Initial work on cortical slices using time-lapse imaging revealed that exposure to an excess of serotonin significantly decreased the migratory speed of INs and PNs.[17,61] In addition, subtle alterations in the laminar distribution of PNs and INs were observed at birth in SERT knockout mice, a widely used model of serotonin excess.[17,61,62] These data further support the notion that an excess of serotonin could affect the migration of different subtypes of cortical neurons *in vivo*. A first indication that 5-HT6R could be involved in cortical neuron migration came from the observations that application of a 5-HT6R agonist to cortical slices could mimic the negative effects of high levels of exogenous serotonin on the migration of INs and PNs.[17,61] In addition, the deleterious impact of high concentrations of serotonin on IN migration was partially reversed by application of a 5-HT6R antagonist. The 5-HT6R is expressed in the VZ, IZ, and cortical plate of the developing cortex and in different subtypes of cortical neurons including cortical INs and upper-layer PNs.[9] To determine whether 5-HT6R controls neuronal migration *in vivo*, 5-HT6R gain- and loss-of-function experiments were performed using *in utero* electroporation targeting the ventricular zone of the dorsal pallium at E14.5.[9] This method has been widely used to specifically target and genetically manipulate PNs. Strikingly, 5-HT6R knockdown induced persistent migratory defects, resulting in a large fraction of PNs abnormally located in deep cortical layers and white matter, whereas 5-HT6R overexpression induced only minor effects on neuronal positioning.[9] Dynamic time-lapse imaging revealed that 5-HT6R knockdown affected both the transition phase from a multi- to a bipolar morphology as well as the process of glia-guided locomotion (Figure 3A). Cdk5 and its activator p35 are key regulators of cortical neuron migration, and Cdk5 loss-of-function induces severe PN migratory defects that partially phenocopy neuron-positioning alterations caused by 5-HT6R knockdown.[63−67] In line with the ability of 5-HT6Rs to constitutively activate Cdk5 signaling, Cdk5 and p35 over-expression rescued the 5-HT6R knockdown migratory phenotype.[9] Furthermore, 5-HT6R gain-of-function in neuroblastoma-glioma cells significantly increased the phosphorylation state of Cdk5 substrates required for neuronal migration, including focal adhesion kinase (FAK at $Ser^{732}$) and microtubule-associated protein doublecortin (DCX at $Ser^{297}$)[9,68,69] (Figure 3B). Conversely, 5-HT6R knockdown in primary embryonic cortical neurons expressing native 5-HT6R significantly reduced phosphorylation of FAK and DCX.[9] In addition to DCX and FAK, Cdk5 phosphorylates the lissencephaly 1 binding protein Ndel1,[70] which controls nucleokinesis by regulating the dynein complex (Figure 3B). Interestingly, Cdk5 also phosphorylates disheveled-axin domain containing-1 (Dixdc1 at $Ser^{250}$), which forms a complex with Ndel and disrupted in schizophrenia 1 (Disc1) to control the polarity switch from the multi- to bipolar morphology.[71] Other Cdk5 substrates required for neuronal migration and


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cytoskeleton dynamics have been identified, including the Cdk inhibitor protein $p27^{kip}$, at $Ser^{10}$,[72] and Neurabin II, at $Ser^{9573}$ (Figure 3B). Whether 5-HT6R regulates the phosphorylation state of these additional Cdk5 downstream targets remains to be established. The precise molecular mechanisms that allow 5-HT6R to regulate Cdk5 activity during PN migration also need to be further investigated. The physical association of Cdk5 with 5-HT6R expressed at the membrane surface of migrating neurons may facilitate the interaction between Cdk5 and its membrane-bound activator, p35. As previously mentioned, serotonin-induced activation of 5-HT6R did not appear to modify the binding of Cdk5 to 5-HT6R,[10] suggesting that expression of 5-HT6R may regulate the Cdk5 pathway through an agonist-independent mechanism. However, one cannot rule out that agonist binding to 5-HT6Rs and the resulting receptor conformational changes further increase Cdk5 activity, notwithstanding their lack of effect upon 5-HT6R/Cdk5 interaction. Taken together, these recent findings indicate that 5-HT6R could operate as an upstream activator of Cdk5 to control PN migration.

## ■ ROLE OF 5-HT6R IN NEURITE GROWTH

The ability of 5-HT6R to engage signaling pathways known to play a pivotal role in dendritic growth/maturation and synapse morphogenesis (i.e., Fyn, mTOR and Cdk5) suggests that it might also control key events in the establishment of neuronal circuitry and connectivity downstream to migration. A role of 5-HT6R in neurite growth was initially shown in neuroblastoma-glioma NG108-15 cells, a commonly used model to investigate neuronal differentiation. Expression of 5-HT6R in NG108-15 cells induced profound morphological changes of the cells such a marked increase in neurite length,[10] similar to those observed after a treatment with dexamethasone plus cAMP, a cocktail classically used to differentiate NG108-15 cells.[74] Moreover, morphological changes elicited by 5-HT6R expression were accompanied by the expression of functional voltage-gated $Ca^{2+}$ channels, a hallmark of NG108-15 cell differentiation.[75] Reminiscent of 5-HT6R/Cdk5 association, the morphogenic effects induced by 5-HT6R were agonist-independent and prevented by antagonists, suggesting a role for Cdk5 signaling. Consistent with this hypothesis, neurite growth of NG108-15 cells elicited by 5-HT6R expression was strongly reduced by pharmacological or genetic inhibition of Cdk5.[10] Notably, 5-HT6R expression also promoted, via a Cdk5-dependent mechanism, neurite growth in primary hippocampal and striatal neurons, as assessed after 1 day *in vitro* (DIV), as well as in cultured explants prepared from the same brain regions, indicating that native 5-HT6Rs can promote neurite growth not only in dissociated neurons but also in organized brain tissue.[10] Moreover, 5-HT6Rs appear to specifically influence neurite extension at that stage of neuronal differentiation, as they did not affect the number of primary dendrites or the complexity of the dendritic tree. Nonetheless, a recent study showed that overexpressing 5-HT6Rs in embryonic cortical neurons reduces the complexity of dendritic arbor, as assessed after 12 DIV, an effect likely resulting from the abnormal growth of the cilia of these neurons.[76] Collectively, these findings suggest that 5-HT6R expression profoundly affects neuronal differentiation and morphology and that it induces contrasting effects upon neurite extension and the complexity of the dendritic tree at different stages of neuronal differentiation. The impact of native 5-HT6Rs on dendrite growth and arborization and the establishment of neuronal connectivity during brain development remains to be established. An important issue will be to discriminate the effects of the receptor on neurite growth from those on migration. Strategies based on gain- or loss-of-function of 5-HT6R at precise stages of brain development would certainly provide valuable information. Likewise, although *in vitro* studies suggest that neurite growth elicited by 5-HT6R expression is independent of mTOR,[10] the precise role of this key regulator of dendrite growth and branching during development remains to be clarified *in vivo*.

Cdk5 can modulate the activity of numerous targets, including proteins that control the dynamics of the cytoskeleton such as small GTPases of the Rho family (RhoA, Rac1, and Cdc42).[40] Of these, only Cdc42 was activated by 5-HT6R expression via a Cdk5-dependent mechanism, and expression of a Cdc42 dominant-negative mutant prevented 5-HT6R-elicited neurite growth both in NG108-15 cells and primary neurons.[10] These findings identify Cdc42 as a downstream effector of neurite growth elicited by the 5-HT6R/Cdk5 complex, reminiscent of its implication in neurite growth of hippocampal neurons induced by BDNF, an effect mediated by Cdk5-mediated phosphorylation of the BDNF receptor, TrkB.[77] However, contrasting with the morphogenic effects of 5-HT6Rs, BDNF increased the number of primary dendrites but did not affect neurite extension. This suggests that neurotransmitter and growth factor receptors, such as 5-HT6R and TrkB, might contribute to the specification of the effects of Cdk5 and Cdc42 on the morphology of the dendritic tree.

Several issues remain to be explored regarding the modulation of neuronal differentiation by the 5-HT6R/Cdk5 complex. An important one is the spatial and temporal regulation of Cdk5 activity by 5-HT6Rs during brain development. The elaboration of a Cdk5 biosensor to detect its activity in cells and tissues would certainly be helpful. The precise mechanisms that control 5-HT6R/Cdk5 interaction, including the potential influence of other receptor partners and the cellular events downstream of 5-HT6R/Cdk5/Cdc42 pathway, also warrant further exploration. As already mentioned, the regulatory role of Cdk5 during brain development extends to synapse formation. Interestingly, interactomic screens identified WAVE1, a Cdk5 substrate known to control dendritic spine morphogenesis,[41,42,77−79] as a 5-HT6R-interacting protein.[10] Whether 5-HT6R-mediated Cdk5 signaling controls synaptogenesis in addition to neuron migration and shaping remains to be investigated.

## ■ ROLE OF 5-HT6R IN NEURODEVELOPMENTAL DISORDERS

Schizophrenia is a disease that generally has its onset during late adolescence or early adulthood, typically between ages 18 and 25, but signs of dysfunction start much earlier in life during the prodromal phase, suggesting that brain development might be affected. The hypothesis of a neurodevelopmental origin is also supported by accumulating evidence from genetic studies indicating that many structural genomic variants associated with schizophrenia affect genes involved in neuronal proliferation and migration, dendritic arborization, and spine morphogenesis.[80−83] As previously emphasized, some of these processes are finely controlled by 5-HT6Rs, but whether developmental abnormalities observed in schizophrenia results from deregulation of 5-HT6Rs and whether drugs acting at 5-


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HT6Rs can correct some of these abnormalities and, thus, be used as disease modifiers remain to be established.

One of the most debilitating problems for patients with schizophrenia is cognitive decline, with the most prominent symptoms being within the domains of executive function, working memory, inhibitory control, reasoning, and social interactions.[18] These deficits, which severely compromise the quality of life of patients and their social and professional integration, are poorly controlled by currently available treatments. Accordingly, intensive efforts are being made to understand the underlying pathological mechanisms and to identify novel strategies for their alleviation.

Among the mechanisms currently under investigation, converging evidence implicates disruption in frontocortical GABAergic transmission, which itself might reflect defects in cortical IN migration and maturation.[84] Besides their influence upon IN migration,[17] 5-HT6Rs have emerged as a promising target for the treatment of cognitive symptoms of schizophrenia.[85−87] 5-HT6 antagonists improve cognition in a broad range of cognitive decline paradigms in rodents, including neurodevelopmental models of schizophrenia, such as neonatal treatment with phencyclidine (PCP) or rearing in social isolation after weaning, which recapitulates in adult animals the cognitive and other behavioral changes characteristic of schizophrenia.[88,89] Furthermore, several antagonists successfully passed phase II of clinical trials for the treatment of cognitive impairment, especially in schizophrenia and Alzheimer's disease, and some of them have been nominated for further development.[90]

The nature of signaling mechanisms mediating the influence of 5-HT6Rs on cognition has long remained unsolved. As outlined above, given the positive influence of the Gs-adenylyl cyclase pathway upon cognition,[18] it was unlikely that the inactivation of this pathway would transduce procognitive effects of 5-HT6 antagonists. The discovery of 5-HT6R coupling to the mTOR pathway[10] was an important step to solve this issue. Indeed, deregulation of mTOR signaling (mostly, but not systematically, its excessive activation) is increasingly recognized as one key signaling event underlying cognitive impairment in various pathological situations, including rare genetic forms of autism spectrum disorder (ASD) such as tuberous sclerosis[24,25,33,34] and Fragile X syndrome (FXS),[91,92] syndromes caused by the disruption of PTEN such as Llermitte-Duclos disease and Cowden syndrome,[26,32,54,93] as well as Down's syndrome.[93] Likewise, memory deficits induced by cannabis consumption are mediated by mTOR activation,[94] and, correspondingly, blocking cannabinoid CB1 receptors rescued behavioral deficits and normalized mTOR activity in a mouse model of FXS.[91] Finally, a recent study showed that a nonphysiological increase in mTOR signaling mediates cognitive and affective deficits caused by the knockdown of *Disc1*, a major gene implicated in schizophrenia, in adult-born dentate granule neurons.[95] In line with these findings, an increase in mTOR activity was detected in the prefrontal cortex of adult rats treated at the neonatal stage with phencyclidine or reared in social isolation.[11] Furthermore, this enhanced mTOR signaling was abolished by 5-HT6 antagonists, and rapamycin, like 5-HT6 antagonists, rescued the deficits in social cognition and episodic memory observed in these developmental models of schizophrenia.[11] These observations show that 5-HT6Rs not only control key developmental processes altered in schizophrenia but also are the trigger of intraneuronal signaling underlying the currently untreatable and strongly debilitating associated cognitive deficits observed at later stages of the disease. They also suggest that mTOR inhibitors, like 5-HT6 antagonists, might be used to alleviate cognitive impairment in patients with schizophrenia. In addition, 5-HT6 antagonists might be evaluated in other psychiatric disorders of neurodevelopmental etiology such as ASDs and Down's syndrome. Finally, it would be interesting to determine in rodent models of schizophrenia whether administration of 5-HT6 antagonists at early developmental time points could prevent the emergence of cognitive and episodic memory defects at the adult stage.

Much remains to be done to elucidate the molecular and synaptic mechanisms underlying the sustained, 5-HT6R-dependent mTOR activation measured at the adult stage in neurodevelopmental models of schizophrenia. Several hypotheses have to be explored, such as an increase in 5-HT release in PFC resulting from changes in network connectivity occurring at critical periods of postnatal brain development and persisting in adulthood, as well as the alteration of 5-HT6R functional status and of its coupling properties. It is also important to establish in these models the temporal patterns of 5-HT6R-elicited mTOR activation and, in the case of early onset, its involvement in aberrant developmental processes occurring during childhood and adolescence in schizophrenia. Finally, the mechanisms whereby excessive mTOR activation, under the control of 5-HT6Rs, perturbs cognition in schizophrenia remain to be established. Several studies have shown that deregulation of mTOR can affect both long-term potentiation and long-term depression in ASD.[34,36,96−99] Deciphering the impact of 5-HT6R-dependent mTOR hyperactivation upon synaptic transmission and synaptic plasticity in the prefrontal cortex may certainly help to understand the synaptic mechanisms mediating the detrimental influence of 5-HT6R on cognition and the cognitive deficits in schizophrenia.

## ■ CONCLUSIONS

5-HT6R has traditionally been viewed as a Gs-coupled GPCR displaying constitutive activity at Gs signaling. Two-hybrid screens and proteomics approaches have greatly extended our knowledge of the various intracellular signaling pathways controlled by 5-HT6R. This has led to the discovery that 5-HT6Rs engage key developmental pathways involved in neuronal circuit formation such as Fyn, Jab1, mTOR, and Cdk5. Recent work aimed at dissecting cellular processes involved in neuronal development revealed that 5-HT6Rs regulate a variety of steps involved in neural circuit assembly, including neuronal migration and neurite outgrowth. Interestingly, expression of 5-HT6Rs appears to be sufficient to control these cellular processes by activating the Cdk5 pathway largely through a ligand-independent mechanism. The precise molecular partners that allow 5-HT6Rs to constitutively activate the Cdk5 pathway remain to be identified but involve the recruitment of the membrane-bound Cdk5 activator p35. In addition, preliminary data suggest that Cdk5 activity can be controlled by pharmacological manipulation of 5-HT6R, but the behavioral consequences of this remain to be established. In contrast, activation or blockade of 5-HT6R through ligand binding has been shown to control the activity of the mTOR pathway and modulate the emergence of psychiatric-relevant behavioral phenotypes. Taken together, recent work in the field has identified 5-HT6R as being a key regulator of a variety of molecular pathways and cellular processes required for the


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precise assembly of neural circuits, thus opening new insights into its role in psychiatric-related phenotypes.

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