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***Title page**

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Non-standard abbreviations used: AD, Alzheimer's disease; ASD, Autism spectrum disorder; AIS, Axonal Initial Segment; Cdk, Cyclin-dependent kinase; DR, dietary restriction; GPCR, G protein-coupled receptor; GPRIN1, G Protein-Regulated Inducer of Neurite Outgrowth 1; Jab1, Jun activation domain-binding protein-1; MAP1B-LC1, light chain 1 subunit of MAP1B protein; mTOR, mechanistic Target Of Rapamycin; mTORC1, mTOR complex 1; NF1, Neurofibromatosis type 1; Nova-1, Neuro-oncological ventral antigen 1; PCP, phencyclidine; Rheb, Ras homolog enriched in brain; SNX14, Sorting Nexin 14; TLE, Temporal lobe epilepsy.

***Abstract**

The serotonin $(5\text{-HT})_6$ receptor is a Gs-coupled receptor exclusively expressed in the central nervous system. Highest receptor densities are found in brain regions implicated in mnemonic functions where the receptor is primarily but not exclusively located in the primary cilium of neurons. The 5-HT_6 receptor continues to raise particular interest for neuropharmacologists, given the pro-cognitive effects of antagonists in a wide range of cognitive impairment paradigms in rodents and human. The 5-HT_6 receptor also finely controls key neuro-developmental processes including neuron migration and differentiation. However, its influence upon neurodevelopment and cognition is not solely mediated by its coupling to the Gs-adenylyl cyclase pathway, suggesting alternative signal transduction mechanisms. This prompted studies aimed at characterizing the receptor interactome that identified 125 candidate receptor partners, making the 5-HT_6 receptor one of the G protein-coupled receptors with the most extensively characterized interactome. These studies showed that the receptor localization at the plasma membrane and, consequently, its signal transduction, are finely modulated by several receptor partners. They demonstrated that prefrontal 5-HT_6 receptors engage the mTOR pathway to compromise cognition in neurodevelopmental models of schizophrenia, and a role of the 5-HT_6-mTOR pathway in temporal epilepsy. Finally, they revealed that the receptor activates Cdk5 signaling in an agonist-independent manner through a mechanism involving receptor phosphorylation by the associated Cdk5 and highlighted its key role in the migration of neurons and neurite growth. These new receptor-operated signaling mechanisms should be considered in the future development of drugs acting on 5-HT_6 receptors.

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Key words: serotonin, 5-HT$_6$ receptor, interactome, constitutive activity, cognition, neurodevelopment

1. Introduction

Serotonin (5-hydroxytryptamine, 5-HT) is certainly one of the most popular neurotransmitters in the world. There are likely two reasons behind this popularity: 1) the positive influence upon mood of serotonin that is often considered as a major contributor of wellbeing and happiness; 2) widely prescribed antidepressants, such as the famous serotonin reuptake inhibitor fluoxetine (Prozac™), target the serotonergic system. This popularity of serotonin has recently been illustrated in France by the success of the short story entitled “Serotonin” published by one of the most “à la mode” French authors, which describes the depressing and decadent life of a French farmer (Houellebecq, 2019).

From the neuropharmacologist point of view, 5-HT continues to raise a great interest, because it provides numerous possibilities of clinical interventions, mainly but not exclusively in the central nervous system. Though the number of serotoninergic neurons is rather small (350,000 in human) (Hornung, 2003), serotonin is implicated in numerous physiological functions such as eating, thermoregulation, cardiovascular functions, locomotion, reproduction, sleep, pain, learning and memory, cognition, aggressiveness, response to stress, emotions, reward and mood. Deregulation of those functions leads to major pathologies such as anxiety (Kahn et al., 1988), depression (Nemeroff and Owens, 2009), anorexia (Jean et al., 2007), headache (Goadsby, 2007), insomnia (Thase, 1999), schizophrenia (Bleich et al., 1988; Meffre et al., 2012; Rasmussen et al., 2011), Alzheimer’s disease (Cirrito et al., 2011; Claeysen et al., 2015) and cognitive dysfunction associated with the majority of these disorders. The wide impact of serotonin on brain functions has been attributed to the extensively branched serotonergic axons that innervate almost all brain nuclei (Charnay and Leger, 2010) and its ability to activate a plethora of receptors: 14 subtypes belonging to seven families (5-HT_1-5-HT_7) have been identified in human (Bockaert et al., 2010a; Millan et al., 2008). All but the 5-HT_3 receptor belong to the G protein-coupled receptor (GPCR) superfamily. The diversity of functions modulated by each 5-HT GPCR subtype not only reflects its capabilities to engage different signal transduction mechanism (Millan et al., 2008), but also its propensity to form complexes with additional proteins,

including proteins involved in non-conventional (G-independent) signaling (Marin et al., 2012).

Among the different 5-HT receptor subtypes, the 5-HT_6 receptor has emerged as one particularly promising target for the treatment of cognitive deficits of various neuropsychiatric diseases such as Alzheimer's disease and schizophrenia, consistent with its highest expression in regions involved in cognitive functions (Codony et al., 2011; de Bruin and Kruse, 2015; de Jong and Mork, 2017). 5-HT_6 receptor antagonists improve cognition in a wide range of preclinical models of cognitive impairment and preliminary clinical studies demonstrated pro-cognitive effects in human. The beneficial effect of antagonists is generally attributed to the blockade of 5-HT_6 receptors located on GABAergic neurons and the relief of GABAergic inhibition of glutamatergic and cholinergic transmissions (Codony et al., 2011; de Jong and Mork, 2017). The presence of 5-HT_6 receptors on glutamatergic neurons introduced a more complex picture (de Jong and Mork, 2017; Helboe et al., 2015), and suggests that 5-HT_6 receptor ligands might affect the excitatory/inhibitory balance within local networks. 5-HT_6 receptors also raise particular interest for the treatment of anxiety and depression as well as eating disorders, but why both agonists and antagonists exert anxiolytic- and antidepressant-like effects and reduce food intake remains to be elucidated. Another important feature of the 5-HT_6 receptor is its early expression during brain development and data suggest that 5-HT_6 receptors mediate defects in brain development induced by an excess of serotonin (Dayer et al., 2015; Riccio et al., 2009). In fact, several studies revealed that the receptor controls key cellular events involved in neural circuit formation such as neuronal migration and neurite growth (Duhr et al., 2014; Jacobshagen et al., 2014).

Characterizing 5-HT_6 receptor-operated signal transduction is prerequisite to fully understand their pathophysiological roles and the therapeutic effects of 5-HT_6 receptor ligands. The 5-HT_6 receptor is canonically coupled to the Gs-adenylyl cyclase pathway (Sebben et al., 1994). As for many GPCRs, 5-HT_6 receptor stimulation also induces activation of the Erk1,2 MAP kinase pathway (Yun et al., 2007). As these pathways generally have a positive

influence upon cognition, it is unlikely that their inhibition by 5-HT_6 receptor antagonists would account for the pro-cognitive effects of these drugs. Likewise, activation of the Gs-adenylyl cyclase pathway only partially accounted for the receptor effects upon neuronal migration (Riccio et al., 2009). Collectively, these observations suggested the existence of alternative coupling mechanisms and prompted several studies based on two-hybrid screens or affinity purification coupled to mass spectrometry (AP-MS) proteomic strategies aimed at characterizing receptor interacting proteins. This review will describe the current knowledge on the 5-HT_6 receptor interactome and its role in receptor signal transduction and associated pathophysiological processes.

2. The 5-HT_6 receptor interactome: one of the most-extensively characterized GPCR interactomes

The Src family non-receptor protein tyrosine kinase Fyn was the first 5-HT_6 receptor interacting protein discovered by the laboratory of Hyewhon Rhim in 2006 thanks to a yeast two-hybrid screen using the receptor's C-terminal domain as bait (Yun et al., 2007). The interaction between full-length Fyn and 5-HT_6 receptor was further demonstrated in transfected cells and rat brain by co-immunoprecipitation.

Using a similar strategy, the same group of investigators then demonstrated an association of the receptor with Jun activation domain-binding protein-1 (Jab1) (Yun et al., 2010), the light chain 1 (LC1) subunit of MAP1B protein (MAP1B-LC1), a microtubule-associated protein highly expressed in the brain (Kim et al., 2014) and, more recently, Neuro-oncological ventral antigen 1 (Nova-1), a neuron-specific RNA binding protein that regulates RNA splicing (Kim et al., 2019) (Table 1). All three proteins were found to co-immunoprecipitate with native receptors expressed in the brain and to interact with the receptor C-terminal domain, suggesting that 5-HT_6 receptors might recruit a number of protein partners in a dynamic manner. Corroborating this hypothesis, AP-MS proteomics screens aimed at identifying 5-HT_6 receptor interacting proteins in a more global manner identified several dozens of putative partners (Duhr et al., 2014; Ha et al., 2015; Meffre et al., 2012). Due to the lack of 5-HT_6 receptor antibody providing immunoprecipitation yields compatible with the

identification of co-immunoprecipitated proteins, these studies were based on immunoprecipitation of recombinant, epitope-tagged receptors expressed in cell lines or pull-downs performed with the C-terminal domain or the intracellular loop (i)3 of the receptor as baits, in line with previous work that identified these domains as critical sites for the recruitment of GPCR interacting proteins and their coupling to G proteins, respectively. Globally, they demonstrated that the receptor recruits several proteins of the mechanistic Target Of Rapamycin (mTOR) pathway, a network of protein including the Cyclin-dependent kinase (Cdk)5 and some of its regulators and substrates known to control the dynamics of actin cytoskeleton, and Sorting Nexin 14 (SNX14), a member of the sorting nexin family involved in protein sorting and vesicular trafficking (Duhr et al., 2014; Ha et al., 2015; Meffre et al., 2012).

Soon after its cloning, the 5-HT_6 receptor was found in cilia-like processes by electron microscopy and peroxidase staining (Brailov et al., 2000; Hamon et al., 1999). Further studies confirmed ciliary localization of endogenously expressed receptors and their co-localization with adenylyl cyclase 3 (AC3), a specific marker of the primary cilium (Brodsky et al., 2017). Nevertheless, the precise characterization of ciliary *vs.* extraciliary localization of 5-HT_6 receptors has so far been hampered by the lack of antibodies allowing detection of low amounts of endogenous receptor and the fact that expression of recombinant receptors leads to ectopic localization outside the primary cilia (Lesiak et al., 2018). This issue was addressed by the generation of knock-in mice expressing GFP-tagged 5-HT_6 receptor (5-HT_6-GFP mice) (Deraredj Nadim et al., 2016). As shown on Figure 1, GFP immunostaining of hippocampal neurons from 5-HT_6-GFP mice not only confirmed the predominant expression of the 5-HT_6 receptor in the primary cilium where it is co-localized with the cilium marker Arl13b, but also revealed a substantial receptor expression in the soma, dendrites and dendritic spines, indicative of both ciliary and extraciliary localizations of endogenously expressed receptors. The ciliary localization of the 5-HT_6 receptor depends on a five amino-acid sequence located in the third intracellular loop (i3) of the receptor which, when recognized by specialized sorting complexes, allows for the crossing of the preciliary barrier

(Berbari et al., 2008; Nachury et al., 2010). However, mutation of this motif only partially prevented the receptor's ciliary localization, suggesting a more complex regulation of its trafficking (Brodsky et al., 2017).

In a recent study, Kohli *et al.* took advantage of the predominant localization of the 5-HT_6 receptor in the membrane of the primary cilium to target APEX2, an enzyme that induces the biotinylation of proteins within a radius of 20 nm in the presence of biotin-phenol, to cilia membrane in order to identify ciliary membrane-associated proteins following their affinity purification on streptavidin beads (Kohli et al., 2017). They also stably expressed a 5-HT_6-APEX-GFP construct in a ciliated cell line and immunoprecipitated the receptor with a GFP antibody in order to characterize the 5-HT_6 receptor interactome specifically in the primary cilium. Most of the proteins identified in this study were actin-binding proteins and differed from the previously identified 5-HT_6 receptor partners, suggesting that the receptor associates with different sets of proteins depending on its localization in primary cilia *vs.* other cell compartments. Intriguingly, the number of common proteins identified in the different proteomic screens is rather limited. This might result from the non-exhaustive identification of affinity-purified proteins in one study (Ha et al., 2015) and the different cell models (HEK-293 and NIH3T3) and approaches (co-IP *vs. in vitro* pull-down assays) used in the other ones (Duhr et al., 2014; Kohli et al., 2017; Meffre et al., 2012). The generation of knock-in mice expressing GFP-tagged 5-HT_6 receptor (Deraredj Nadim et al., 2016) will certainly provide a unique opportunity to directly identify receptor partners in an authentic context and at various development stages.

Collectively, previously published studies of the 5-HT_6 receptor interactome identified 125 putative receptor partners (Table 1). Analysis of Gene Ontology (GO) terms overrepresented in this comprehensive dataset to determine the major biological processes and functions within the 5-HT_6 receptor interactome revealed an overrepresentation of proteins related to "actin cytoskeleton organization" (46 proteins), "post-synapse organization" (37 proteins), "TOR signaling" (32 proteins), "receptor-mediated endocytosis" (30 proteins), "regulation of endocytosis" (17 proteins) and "lamellipodium organization" (Figure 2, Tables 1 and 2). Other

overrepresented GO terms are “regulation of phosphoprotein phosphatase activity”, “regulation of nucleocytoplasmic transport”, “regulation of terminal button organization” and “Rac protein signal transduction”. Though a limited number of interactions have so far been functionally validated (see Table 1), studies indicate a critical influence of some interacting proteins upon the 5-HT_6 receptor trafficking and plasma membrane localization and, accordingly, efficacy of signal transduction, while other partners stabilize the receptor in active conformations able to transduce signals in absence of agonist, as it will be described in the next chapter.

3. From the modulation of canonical pathways to the engagement of novel signaling pathways

3.1 Modulation of canonical pathways

Association of the 5-HT_6 receptor with Fyn, or Jab1 or Map1B-LC1 increases receptor expression at the plasma membrane likely by preventing its internalization (Kim et al., 2014; Yun et al., 2010; Yun et al., 2007) (Figure 3A and B), whereas receptor interaction with SNX14 and Nova-1 promotes its endocytosis and degradation (Ha et al., 2015; Kim et al., 2019) (Figure 3C and D). Given the large number of receptor interacting proteins identified in the different interactomics screens that exhibit “receptor endocytosis and recycling” or “regulation of endocytosis” GO annotations (Table 1), it is likely that additional partners control receptor plasma membrane expression and stability.

Corroborating their contrasting effects upon receptor trafficking, Fyn, Jab1 and Map1B-LC1 enhance receptor coupling to G proteins and signal transduction (Kim et al., 2014; Yun et al., 2010; Yun et al., 2007), whereas Nova-1 and SNX14 behave as negative regulators of receptor-operated signaling pathways (Ha et al., 2015; Kim et al., 2019). SNX14 is also able to bind to Gαs proteins *via* its putative RGS binding domain, thus inhibiting receptor-operated Gs signaling (Figure 3D). SNX14 might thus negatively regulate 5-HT_6 receptor signaling by promoting both endocytic receptor degradation and Gαs sequestration (Ha et al., 2015).

The data also indicate that some of the identified interactions not only regulate the receptor

functional activity but also affect the function of the partners. 5-HT_6 receptor activation promotes Fyn phosphorylation on Tyr^{401} and Erk1,2 MAP kinase activation through a mechanism dependent of both Fyn activation and 5-HT_6 receptor/Fyn interaction (Yun et al., 2007) (Figure 3A). 5-HT_6 receptor stimulation reduces its interaction with Jab1. This leads to Jab1 translocation to the nucleus and its association with c-Jun, thus stimulating the binding of c-Jun and Jun D to AP-1 sites and their transcription factor activity (Yun et al., 2010) (Figure 3B). These findings suggest that 5-HT_6 receptors can regulate gene expression *via* Jab1. A more recent study showed that 5-HT_6 receptor expression as well as the activation of the Jab1/phospho-c-jun pathway are increased in a rat model of status epilepticus induced by pilocarpine injection (Liu et al., 2019). Treatment of the epileptic rats with the 5-HT_6 receptor antagonist SB271046 normalized the Jab1/phospho-c-jun pathway activity, reduced the frequency of spontaneous recurrent seizures and reversed the associated learning and memory impairments (Liu et al., 2019). This strongly suggests an important role of the Jab1/phospho-c-jun pathway, under the control of 5-HT_6 receptors, in status epilepticus

Over-expression of the 5-HT_6 receptor promotes Nova-1 translocation from the nucleus to the cytosol and thus inhibits its splicing activity (Kim et al., 2019) (Figure 3C). Again, this effect is likely mediated by the physical interaction between 5-HT_6 receptor and Nova-1, as expression of a truncated version of the receptor deleted of the C-terminal domain did not affect Nova-1 localization and splicing activity.

3.2. Constitutive activity

An important feature of the 5-HT_6 receptor is its high level of constitutive activity at Gs signaling, which was first established for recombinant receptors expressed in cell lines (Kohen et al., 2001) and then for native receptors in primary cultured neurons and the mouse brain (Deraredj Nadim et al., 2016). In light of previous findings indicating a possible influence of GPCR interacting proteins on their constitutive activity (Ango et al., 2001; Bockaert et al., 2010b), Deraredj-Nadim *et al.* focused on the role of receptor interaction with Neurofibromin, a Ras GTPase-activating protein (Ras-GAP) encoded by the tumor suppressor gene *NF1* known to be involved in adenylyl cyclase activation by various GPCRs

(Deraredj Nadim et al., 2016). Mutations in the *NF1* gene are responsible for Neurofibromatosis type 1 (NF1), a genetic disorder characterized by skin pigmentation, benign skin tumors called neurofibromas, low-grade tumors of the central and peripheral nervous systems and, in some patients, learning and attention deficits (Trovo-Marqui and Tajara, 2006). Silencing Neurofibromin expression in HeLa cells and primary striatal neurons strongly reduced 5-HT_6 receptor constitutive activity at Gs signaling (Deraredj Nadim et al., 2016). Likewise, the level of receptor's constitutive activity (assessed by measuring phosphorylated CREB and cAMP levels) was strongly reduced in the brain of *Nf1* heterozygous mice (*Nf1*$^{+/-}$, NF1 model), compared to WT mice. Furthermore, treatment of brain micro-discs from *Nf1*$^{+/-}$ mice with SB271046, a 5-HT_6 receptor inverse agonist, did not further decrease this reduced cAMP level, whereas this compound did inhibit basal cAMP formation in micro-discs from WT mice brain. Finally, agonist-independent activation of the receptor in cultured cells was strongly reduced by an interfering peptide corresponding to the 22 C-terminal amino acid residues of the receptor that disrupts 5-HT_6 receptor/Neurofibromin interaction or by *NF1* mutations identified in NF1 patients that are located in Neurofibromin PH domain and affect its recruitment by the receptor (Deraredj Nadim et al., 2016). Collectively, these findings demonstrate a key influence of the 5-HT_6 receptor/Neurofibromin physical interaction on the constitutive activity of the receptor.

Another process that may influence 5-HT_6 receptor constitutive activity is its localization in the primary cilium. Primary cilia are microtubule-based structures found on almost every cell type (Wheatley et al., 1996). They are dynamic structures that can assemble, disassemble and reassemble in the cells, depending on the cell cycle (Lepanto et al., 2016a; Lepanto et al., 2016b). They have numerous specialized roles, including light or odor sensing, which are defined by the identity of the receptors and signaling proteins they contain. Disruption in cilia function through the mutations of genes involved in their formation or function are linked to diseases termed ciliopathies (Reiter and Leroux, 2017). Though the 5-HT_6 receptor is the only serotonin receptor targeted to the cilium and its ciliary localization has been known for two decades, little is known about the role of its targeting to the cilia. In an elegant series of

experiments based on the use of a biosensor allowing a direct visualization of changes in cAMP concentration in the primary cilium in combination with strategies to discriminate cAMP signal in the cilium and the cell body, Jiang et al. recently demonstrated that 5-HT_6 receptors expressed in the ciliary membrane display a reduced level of constitutive activity, when compared to receptors located outside the cilium (Jiang et al., 2019). These findings together with the demonstration that native receptors exhibit constitutive activity strongly support that part of endogenously expressed receptors are located outside the cilium. The mechanism whereby 5-HT_6 receptor constitutive activity is abolished in the cilium remains to be fully elucidated. Treatment of cells harboring ciliary localization of 5-HT_6 receptors with an agonist of smoothened receptors to activate the Hedgehog (Hh) signaling pathway restored receptor constitutive activity (Jiang et al., 2019), suggesting that the absence of Hh signaling might affect receptor coupling to Gs, or the ciliary expression of G protein subunits or signaling molecules such as adenylyl cyclase. Other explanations for the reduced constitutive activity of 5-HT_6 receptors in the primary cilium include the distinct lipid composition of the ciliary membrane, which may impair its ligand-independent activation or the absence of receptor partners essential for constitutive activity. Notably, Neurofibromin was not identified in the proteomic screen assessing partners of receptors specifically localized in the cilia (Kohli et al., 2017). This suggests that the disruption of 5-HT_6 receptor/Neurofibromin interaction in the cilia might be one of the causes of the absence of constitutively active receptors in this compartment.

3.3. Engagement of novel signaling pathways

3.3.1. mTOR pathway

As shown in Figure 4 and Table 2, the 5-HT_6 interactome shows a remarkable enrichment in proteins of mTOR signaling pathway (25% of identified proteins are encoded by genes with the “TOR signaling” GO annotation). These include mTOR itself and Raptor, another component of the mTOR complex 1 (mTORC1). These findings suggested a potential engagement of mTORC1 signaling upon 5-HT_6 receptor stimulation that was further established in both cell lines expressing recombinant receptors and rodent brain, especially

in the prefrontal cortex and the striatum, following peripheral administration of a 5-HT_6 receptor agonist (Meffre et al., 2012). Receptor-dependent activation of the mTOR pathway was inhibited by pharmacological inhibitors of PI3K or the expression of a dominant-negative Ras homolog enriched in brain (Rheb) mutant, indicating that it involves the canonical PI3K/Akt/Rheb pathway implicated in mTOR activation by growth factor receptors (Figure 4). Furthermore, it required a physical interaction between the receptor's C-terminal domain and mTOR (Meffre et al., 2012). Interestingly, receptor-operated mTOR activation not only occurs in response to the administration of an agonist, but also in specific pathophysiological conditions. In two neuro-developmental models of schizophrenia, rats treated with the NMDA receptor antagonist phencyclidine (PCP) at the neonatal stage and rats reared in isolation after weaning, a non-physiological mTOR activation was measured specifically in prefrontal cortex, whereas mTOR was not affected in striatal neurons (Meffre et al., 2012). Moreover, administration of a 5-HT_6 receptor antagonist at the adult stage normalized prefrontal mTOR activity level in both models, indicating that it depends on a sustained activation of 5-HT_6 receptors. Likewise, an increased in mTOR activity was measured in the hippocampus and cortex of rats treated with pilocarpine, a model of temporal epilepsy (TLE), which correlated with an increase in 5-HT_6 receptor expression (Wang et al., 2015). An enhanced expression of the 5-HT_6 receptor was likewise found in the temporal cortex of TLE patients (Wang et al., 2015). Furthermore, pre-treatment of pilocarpine-treated rats with the specific 5-HT_6 receptor antagonist SB399885 prevented both the increase in 5-HT_6 receptor expression and mTOR activation in the hippocampus measured after pilocarpine-induced seizures, suggesting a key influence of 5-HT_6 receptors in the overactivation of mTOR occurring in TLE (Wang et al., 2015). Conversely, chronic (8 weeks) dietary restriction (DR) in the mouse induces a decrease in 5-HT_6 receptor expression in the hippocampus and the prefrontal cortex and, correspondingly, a decrease in mTOR activity in these brain areas, compared with mice that received a normal diet (Teng et al., 2019). Further supporting the critical role of 5-HT_6 receptors in the maintenance of a basal level of mTOR activity in the brain of mice fed *ad libitum*, mTOR activation level was strongly reduced in the hippocampus of 5-HT_6 receptor

knock-out mice (5-$HT_6^{-/-}$ mice), compared to wild type (WT) mice, and chronic dietary restriction did not further decrease this reduced mTOR activity level.

3.2.2. Cdk5 pathway

Among the signaling proteins recruited by the 5-HT_6 receptor C-terminal domain, Cdk5 also raised particular attention given its common influence with the receptor upon neuro-developmental processes such as migration (Dayer et al., 2015; Lalioti et al., 2010). Interaction between the full-length receptor and Cdk5 was first validated by co-immunoprecipitation and BRET (Duhr et al., 2014). These experiments showed that 5-HT_6 receptors bind to Cdk5 in an agonist-independent manner but that the interaction is disrupted upon treatment of cells by a 5-HT_6 receptor antagonist. Further experiments showed that Cdk5 bound to the receptor was active and that this activity was inhibited by treatment of cells with an antagonist, which thus behaved as an inverse agonist at Cdk5 signaling. Interesting, a Ser residue closed to Cdk5-binding site in the receptor C-terminal domain (Ser^{350}) was identified as a substrate of Cdk5 bound to the receptor. Furthermore, Ser^{350} phosphorylation was found to be essential to receptor-mediated activation of Cdk5 signaling (Duhr et al., 2014). Collectively, these findings suggest a reciprocal interplay between the 5-HT_6 receptor and associated Cdk5 whereby Cdk5 phosphorylates the Ser^{350} residue, a process allowing agonist-independent activation of Cdk5 by the receptor. They provide the first demonstration of constitutive activity of a GPCR that depends on its phosphorylation by an associated protein kinase. They also indicate that 5-HT_6 receptors not only constitutively activate the canonical Gs-adenylyl cyclase pathway, but also additional, Gs-independent, pathways.

4. Neurodevelopmental roles of the 5-HT_6 receptor interactome

Neurodevelopment is achieved through a succession of tightly regulated processes. Stem cells give birth to neuroblasts, which will migrate to reach their final position. They will then start emitting neurites, which will differentiate to form an axon and multiple dendrites. Finally, the morphogenesis of dendritic spines will allow for the network to become connected. Understanding these processes has become a major challenge, as any alteration affecting

them could result in neurodevelopmental diseases, such as schizophrenia or autism spectrum disorder (ASD). Serotonin has long been known to play key roles in neurodevelopment (Gaspar et al., 2003), and more recently an emphasis has been put on the 5-HT_6 receptor (Dayer et al., 2015; Vitalis et al., 2013). This receptor is expressed very early in development, and unlike other serotonin receptors, it is almost exclusively expressed in the central nervous system. Several studies have shown that the receptor and likely some of its protein partners are involved in every neurodevelopmental step. The role of the receptor in neural circuit formation has been reviewed elsewhere (Dayer et al., 2015). Here, we will focus on the roles of the receptor and its protein partners, with a special emphasis on newly discovered ciliary functions of the receptor.

4.1. Neurogenesis

The level of serotonin in the brain during development has to be precisely regulated, as both an excess or a lack of serotonin have deleterious effects on the proliferation of neural progenitors. For example, depleting serotonin in embryonic mice through genetic or pharmacological approaches results in smaller brain size, suggesting that proliferation is reduced (Vitalis et al., 2013). On the other hand, knock-out mice for the monoamine oxidases MAOA and B, which are involved in the degradation of serotonin, present an excess of serotonin but also show a reduced proliferation of neuronal stem cells (Cheng et al., 2010), which indicates that there is likely a balance point in the concentration of serotonin that has to be maintained for normal brain development. Pointing to a role of the 5-HT_6 receptor in this process, the effects of excess serotonin can be reversed by the inhibition of the 5-HT_6 receptor function, using either specific antagonists or siRNA (Wang et al., 2014), through the restoration of the activation of caspase 3 and 9, reinstating normal apoptotic activity in the developing brain. This suggests that the 5-HT_6 receptor is coupled to anti-apoptotic signaling pathways. Interestingly, Jab1, one of the receptor interacting proteins identified by a yeast two-hybrid screen (Yun et al., 2010), is a component of the Constitutive Photomorphogenic-9 (COP9) signalosome, which can regulate multiple intracellular signaling pathways (Chamovitz and Segal, 2001), including cell protection against apoptosis. The anti-apoptotic

effects of serotonin might thus be mediated, at least in part, by the 5-HT_6 receptor, *via* Jab1 activation.

4.2. Neuronal migration

Serotonin plays an important role in neuronal migration. As for neurogenesis, the impact of serotonin seems to be tightly dependent of its concentration. Serotonin transporter (SERT) knock-out mice, which present high serotonin levels, also display an abnormal distribution of pyramidal neurons in the cortex (Frazer et al., 2015; Riccio et al., 2009; Vitalis et al., 2013). Lowering the serotonin concentration, either pharmacologically or genetically, also results in mispositioning of cortical neurons (Vitalis et al., 2007; Vitalis and Parnavelas, 2003). It has been shown that a low concentration of serotonin (5 µM) can accelerate the migration of neurons from cortical explants, whereas a high concentration (100 µM) slows down the migration of both cortical GABAergic interneurons and pyramidal neurons (Durig and Hornung, 2000). This suggests that a tight regulation of the serotonin level is necessary for correct positioning of cortical neurons. The involvement of the 5-HT_6 receptor has been demonstrated by showing first that this receptor is expressed in these neurons at that developmental stage, and that EMD386088, a specific agonist of the receptor, fully recapitulates the effect of serotonin on migration (Riccio et al., 2011; Riccio et al., 2009; Vitalis and Parnavelas, 2003). Furthermore, delivery of a specific antagonist of the receptor (SB258585) or inhibiting receptor-mediated Gs signaling by treatment with either a PKA inhibitor or a non-hydrolysable analogue of cAMP, reversed the effects of serotonin on migration (Riccio et al., 2009).

Independent of the effect of serotonin, the 5-HT_6 receptor has also been shown to control neuronal migration through a Cdk5-dependent pathway (Jacobshagen et al., 2014). The data show that reducing 5-HT_6 receptor expression or inhibiting the Cdk5 kinase activity results in mispositioning of cortical pyramidal neurons, due to both an inability of the neurons to switch from a multipolar to bipolar morphology and a defect in locomotion. The role of Cdk5 in the multipolar to bipolar transition is well documented (Ohshima et al., 2007a), but this study was the first to demonstrate a link with serotonin and the 5-HT_6 receptor.

It is also interesting to note that in addition to Cdk5, the interactome of the 5-HT_6 receptor includes the Fyn tyrosine kinase (Yun et al., 2007), and several proteins of the mTORC1 pathway (Meffre et al., 2012). These proteins can affect the reelin-dab1 signaling pathway, which controls the precise positioning of neurons (Beffert et al., 2004; Moon et al., 2015; Ohshima et al., 2007a; Ohshima and Mikoshiba, 2002; Ohshima et al., 2007b). As already described, Fyn kinase activity is promoted by the activation of the 5-HT_6 receptor (Yun et al., 2007). However, the exact role of receptor-elicited Fyn signaling in migration remains to be established. The mTORC1 pathway is known to have a role in neuronal migration, dendritic tree formation and dendritic spine morphogenesis (Takei and Nawa, 2014). Deregulation of this pathway results in numerous neurodevelopmental disorders, including some rare genetic forms of ASD, Rett and Down syndromes (Bockaert and Marin, 2015). The impact of the 5-HT_6 receptor-mediated mTOR activation in migration also remains to be elucidated.

4.3. Neurite growth

Once a neuroblast has reached its final destination, it will start emitting neurites, which will eventually form dendrites and the axon of the mature neuron. Depleting serotonin levels alters the length and complexity of dendrites in different areas of the cortex (Durig and Hornung, 2000; Feria-Velasco et al., 2002; Gonzalez-Burgos et al., 1996; Riccio et al., 2011; Riccio et al., 2009; Vitalis et al., 2007) and in the hippocampus (Alves et al., 2002). A role for the 5-HT_6 receptor-Cdk5 complex in neurite growth has been demonstrated in various models including the NG108-15 neuroblastoma cell line, primary neuronal culture and mouse brain explants (Duhr et al., 2014). Of note, the 5-HT_6 receptor interactome also contains the G Protein-Regulated Inducer of Neurite Outgrowth 1 (GPRIN1). GPRIN1 is a protein highly and specifically expressed in the brain, which selectively binds to activated G proteins and promotes neurite extension (Ge et al., 2009; Iida and Kozasa, 2004; Nordman and Kabbani, 2012). Whether GPRIN1 association with the 5-HT_6 receptor contributes to neurite growth remains to be investigated.

4.4. Impact of ciliary 5-HT_6 receptor localization

Primary cilia are observed in developing neurons and participate in every step of neurodevelopment (Park et al., 2019; Youn and Han, 2018). Cilia are present in migrating cortical interneurons and allow them to escape the migration path and to colonize the cortical plate through the Sonic Hedgehog (Shh) and Wnt signaling pathway (Baudoin et al., 2012). They drive the migration of cerebellar granule neurons along glial fibers through the control of myosin II and F-actin dynamics (Trivedi et al., 2014). Primary cilia also participate in neuronal differentiation. They exhibit a highly dynamic behavior regarding both their presence and localization during the differentiation of retinal ganglion cells. Furthermore, cilia disruption experiments revealed their essential role in the proliferation and survival of retinal progenitors, as well as the generation of retinal ganglion cells (Lepanto et al., 2016b). Cilia are also found on mature hippocampal or cortical neurons, where they can play a role in dendritic growth and complexification as well as adult neurogenesis (Guadiana et al., 2013; Kumamoto et al., 2012). The role of primary cilia in neurodevelopment has been extensively reviewed recently (Park et al., 2019; Youn and Han, 2018). Here, we will describe recent data suggesting a key role of 5-HT_6 receptors located in the cilia in neuronal differentiation and morphology.

Pharmacological blockade of 5-HT_6 receptors in cultured striatal neurons results in a shortening of the primary cilia, whereas their stimulation with an agonist shows no effect on cilia morphology, suggesting that the receptor influence on cilia length mainly depends on its constitutive activity (Brodsky et al., 2017). Corroborating these findings, restoring the expression of the receptor in cilia of neurons from 5-$HT_6^{-/-}$ mice increased cilia length in an agonist-independent manner (Lesiak et al., 2018). The effect of further over-expression of the receptor is less clear, with a study showing that it had no additional effect on cilia length (Brodsky et al., 2017), whereas two further studies showed a dramatic increase in length and even branching of the cilia when the receptor is overexpressed (Guadiana et al., 2013; Hu et al., 2017). Additional studies are needed to explain these discrepancies. Further supporting the role of agonist-independent activation of the receptor in cilia extension, expression of a mutated 5-HT_6 receptor insensitive to 5-HT and other agonists but displaying constitutive

activity (5-HT_6^{D106A}) also induced an increase in cilia length (Lesiak et al., 2018). The ability of the 5-HT_6 receptor to control cilia length makes it likely to have an important role in neuronal architecture. Further supporting this hypothesis and corroborating previous observations indicating a key influence of receptor constitutive activity in neurite growth (Duhr et al., 2014), restoration of the 5-HT_6 receptor expression in neurons from 5-$HT_6^{-/-}$ mice increased the average dendrite length, an effect reproduced by the expression of the 5-HT_6^{D106A} receptor mutant (Lesiak et al., 2018). On the other hand, expression of a 5-HT_6 receptor mutant deleted of the Fyn binding site did not affect dendrite length (Lesiak et al., 2018), suggesting that a Fyn-regulated signaling pathway dependent of its recruitment by 5-HT_6 receptors is involved in the regulation of neurite growth. Another study has shown that Src-family kinases such as Fyn can activate the Cdk5 pathway through the induction of the expression of the Cdk5 activator p35 (Kobayashi et al., 2014). Accordingly, 5-HT_6 receptor-mediated activation of Cdk5 signaling to promote neurite growth might involve a dual mechanism involving receptor phosphorylation by associated Cdk5 and Fyn-dependent induction of p35 expression.

Consistent with a role of the ciliary localization of 5-HT_6 receptors in neurodevelopment, a RNA-interference study conducted on 41 genes associated with neurodevelopmental diseases showed that more than half of these genes control cilia length (Marley and von Zastrow, 2012), implying that a dysregulation of cilia length and hence its signaling capacity might be key factors in the development of these pathologies. Further evidence of the role of ciliary 5-HT_6 receptors in neuronal morphology has been obtained in the APP/PS1 mouse model of Alzheimer's disease (AD). In these mice, the expression of the 5-HT_6 receptor is increased in the hippocampus, with a correlated increase in cilia length compared to WT mice, suggesting that cilia elongation in this model might be induced by the upregulation of 5-HT_6 receptor levels (Hu et al., 2017). The enhanced 5-HT_6 receptor expression is also accompanied with an increase in Axonal Initial Segment (AIS) length, a decrease in its distance relative to the soma, the translocation of ankyrin G, a marker of the AIS to the ciliary compartment and a reduction of axonal length (Hu et al., 2017; Wang et al., 2014). Finally,

administration of the selective 5-HT_6 receptor inverse agonist SB271046 to the APP/PS1 mice restored normal cilia length and AIS morphology, which correlated with its ability to prevent cognitive impairment, suggesting that the 5-HT_6 receptor might affect cognition in Alzheimer's disease through the regulation of cilia length and AIS morphology, which is known to have a crucial influence upon neuronal excitability (Yamada and Kuba, 2016).

5. Pathophysiological impact of the 5-HT_6 receptor interactome

5.1 Chemical probes to study the pathophysiological impact of the 5-HT_6 receptor

Over the last two decades, the 5-HT_6 receptor has been the focus of attention of numerous pharmaceutical companies in view of its restricted pattern of expression in the brain and the spinal cord, making it an attractive target. Although this focus has been mainly put on antagonists because of their clear pro-cognitive properties, both agonists and antagonists are considered as potentially valuable therapeutic tools that can also shed light on the receptor's physiological functions.

5.1.1 Agonists

5-HT_6 receptor agonists have been tested for several applications, including nociception, cognitive functions, mood, addiction, obesity and sleep, but their effects remain often controversial. Their therapeutic potential has recently been reviewed in depth (Karila et al., 2015). So, we will mostly illustrate some conflicting data obtained by taking as an example the widely used agonist EMD386088. EMD386088 induces clear-cut pro-nociceptive effects, in contrast to several antagonists (Castaneda-Corral et al., 2009; Godinez-Chaparro et al., 2011; Godinez-Chaparro et al., 2012), while its influence on behavioral flexibility remains still controversial. A recent study has shown that EMD386088 impairs reversal learning (Amodeo et al., 2018), whereas a previous study found no effect of EMD386088 used at a similar dose (Nikiforuk, 2014; Nikiforuk et al., 2011). The authors postulated that this discrepancy was likely due to the difference in the behavioral tests used to assess reversal learning. EMD386088 has also been shown to impair both long-term and short-term working memory (Amodeo et al., 2018; Meneses et al., 2008), an effect that was also established for another

5-HT_6 receptor agonist, WAY181187 (Loiseau et al., 2008). Again, this contradicts the data of another study which showed a significant improvement of working memory in rats treated with EMD386088 or with the agonist E-6801 (Kendall et al., 2011). These conflicting results may be explained by the different models of memories studied (novel object recognition in (Kendall et al., 2011) vs. social recognition and associative learning in the other studies), which would suggest a specific effect of the receptor in different types of memories. Notably, these conflicting results come from studies that have investigated the effect of agonists on healthy animals. When used in models of cognitive impairment, EMD386088 as well as WAY181187 have been shown to improve memory in the same way as antagonists (Bokare et al., 2018; Nikiforuk et al., 2013; Rychtyk et al., 2019). EMD386088 also reverses cognitive deficits in a rat model of schizophrenia (ketamine treatment) (Nikiforuk et al., 2013). This suggests an alteration of a finely-tuned 5-HT_6 receptor signaling in pathological situations which can be normalized by both agonists and antagonists.

5-HT_6 receptor agonists have also been studied in the frame of depression and anxiety. EMD386088 displays anxiolytic and antidepressant effects (Jastrzebska-Wiesek et al., 2014; Nikiforuk et al., 2011), reminiscent of the effects of antagonists. A recent review has suggested that different molecular mechanisms might underlie the anxiolytic like effects induced by both agonists and antagonists (Nikiforuk, 2014).

5.1.2. Antagonists

5-HT_6 receptor antagonists have been extensively studied and there is a large consensus that they have pro-cognitive properties. Numerous studies have shown unequivocal beneficial effects of 5-HT_6 receptor antagonists on cognitive deficits in rodent models of psychiatric and neurodegenerative diseases such as schizophrenia and AD and have been extensively reviewed elsewhere (de Bruin and Kruse, 2015; de Jong and Mork, 2017; Ferrero et al., 2017; Khoury et al., 2018; Lalut et al., 2017). Though 5-HT_6 receptor blockade raised an important hope as symptomatic treatment to improve cognition in AD, the initial enthusiasm was tempered by the recent failure of leading compounds in Phase 3 trials. For instance, the selective and high-affinity 5-HT_6 receptor antagonist Idalopirdine (Lu AE58054)

in conjunction with the acetylcholine esterase inhibitor Donepezil improved cognitive function in patients with moderate AD in a randomized, double-blind, placebo-controlled Phase 2 trial (Wilkinson et al., 2014) but did not show any benefit in these patients after treatment for 6 months in subsequent Phase 3 studies (Atri et al., 2018). This failure might be linked to the reduction in the doses and changes in the dosing regimen from the Phase 2 to the Phase 3 studies. Phase 2 studies investigating another selective 5-HT_6 receptor antagonist, Intepirdine (SB742457), alone or in conjunction with Donepezil, in patients with mild to moderate AD, did not show any efficacy of Intepirdine used as monotherapy but, when added as adjunct to Donepezil, the compound induced a significant improvement of cognition and functional status, when compared with Donepezil treatment alone (Maher-Edwards et al., 2015). However, in a subsequent Phase 3 study, Intepirdine failed to demonstrate a statistically significant benefit in cognition in AD patients who were already taking Donepezil (Axovant Sciences press release, September 26, 2017). A novel 5-HT_6 receptor antagonist, SUVN-502, is currently under clinical investigation in subjects with moderate AD who received either Donepezil or the NMDA receptor antagonist memantine or the combined therapy.

These failures underscore the need of new studies to understand the molecular and cellular mechanisms underlying the pathophysiological influence of the 5-HT_6 receptor. A recent work suggests that the regulation of cilia function by the receptor could impact cognition (Hu et al., 2017). The demonstration of receptor constitutive activity *in vivo* (Deraredj Nadim et al., 2016) also points the importance of designing 5-HT_6 receptor ligands with inverse agonist or neutral antagonist properties. Molecules such as Intepirdine have been shown to have an inverse agonist effect on receptor-operated Gs and Cdk5 signaling (Grychowska et al., 2016; Vanda et al., 2018). Interestingly, Intepirdine displays stronger effects on cognition when compared to the recently designed neutral antagonist CPPQ, but the latter had a higher anxiolytic effect (Grychowska et al., 2016), suggesting that targeting constitutive or evoked activity has a differential impact on behavior and cognition. A partial inverse agonist, which does not alter receptor-mediated Cdk5 signaling but partially inhibits constitutive activity at

Gs signaling displayed pro-cognitive properties, and reversed scopolamine-induced memory deficits, when delivered in conjunction with donepezil (Vanda et al., 2018).

Finally, a focus has also been put on developing multi-target compounds that bind to the 5-HT_6 receptor and other receptors. For example, in an effort to find drugs that could control cognitive symptoms in patients with schizophrenia, dual 5-HT_6 receptor/dopamine D_3 receptor antagonists have been developed (Grychowska et al., 2019; Saavedra et al., 2017). Interestingly, the compound 19 synthetized by Grychowska and colleagues is a neutral 5-HT_6 receptor antagonist as well as a high-affinity D_3 receptor antagonist (Grychowska et al., 2019). This compound displays neuroprotective properties, which are not reproduced by Intepirdine, and is capable of reversing cognitive deficits in PCP-treated mice (schizophrenia model). Compounds combining 5-HT_4 receptor agonist/5-HT_6 receptor antagonist activities have recently been synthesized. The anti-amnesic effect of one of them (used at a dose of 1 mg/kg) has been established in a scopolamine-induced deficit model of working memory (Yahiaoui et al., 2016). More recently, the same group of investigators designed and synthesized a novel multi-target directed ligand combining 5-HT_4 receptor agonist, 5-HT_6 receptor inverse agonist and acetylcholine esterase inhibitor activities that displayed an anti-amnesic effect at a dose of 0.3 mg/kg in the same model of working memory deficit (Hatat et al., 2019). These encouraging preliminary results highlight the potential of multitarget strategies for the treatment of cognitive deficits of psychiatric and neurodegenerative disorders.

5.2. Role of the 5-HT_6 receptor interactome in cognitive deficits

As previously mentioned, 5-HT_6 receptor stimulation promotes mTOR signaling in prefrontal cortex in neurodevelopmental models of schizophrenia (Meffre et al., 2012). In light of previous findings suggesting a deleterious influence of non-physiological mTOR activation in neurodevelopmental disorders, including rare genetic forms of ASD and Down syndrome (Ehninger and Silva, 2011; Troca-Marin et al., 2012), Meffre *et al.* demonstrated that an acute administration of the mTOR pharmacological inhibitor rapamycin prevented the deficits in episodic memory and social discrimination tests in rats treated with PCP at the neonatal

stage or reared in isolation after the weaning, thereby reproducing the effects of 5-HT_6 receptor antagonists/inverse agonists (Meffre et al., 2012). These findings suggested that the upregulation of mTOR associated with prefrontal 5-HT_6 receptors might be involved in cognitive deficits of schizophrenia, which remain poorly controlled by currently available antipsychotics. They also suggested that 5-HT_6 receptor antagonists might be evaluated in genetic ASD forms exhibiting excessive mTOR activation (Ehninger and Silva, 2011). mTOR is a critical signaling node that controls many neuronal functions in response to numerous extracellular signals, including growth factors and neurotransmitters. It is not only essential for the proper structural and functional development of brain circuitry but also has a key influence upon synaptic transmission and synaptic plasticity in the mature brain (Bockaert and Marin, 2015). However, the mechanisms by which over-activation of mTOR signaling induces cognitive impairment remain to be fully elucidated. Excessive mTORC1 activation in ASD causes a decrease in autophagy, an increase in spine density, and a reduction of developmental spine pruning in pyramidal neurons. Alteration of spine formation and maturation and deregulation of specific forms of synaptic plasticity such as long-term depression have also been proposed (Bockaert and Marin, 2015).

As previously mentioned, the neurochemical mechanisms underlying pro-cognitive effects of 5-HT_6 receptor antagonists in preclinical models of AD are quite well characterized. They likely involve the blockade of receptors located on GABAergic interneurons, which in turn reduces interneuron-mediated inhibition of cholinergic and glutamatergic neurons and increases cholinergic and glutamatergic transmissions (Codony et al., 2011). How the reduction of mTOR signaling that is overactivated in the brain of AD patients (An et al., 2003), supports the pro-cognitive effects of 5-HT_6 receptor antagonists remains to be established. It has been suggested that aberrant activation of mTOR contributes to amyloid and Tau pathologies in AD *via* several mechanisms. These include the generation of fibrillar β-amyloid (Aβ) peptide, the increase in Tau phosphorylation at sites related to tauopathies and Tau protein levels, the formation of neurofibrillary tangles, the inhibition of autophagy and of the clearance of pathologically accumulated proteins (Caccamo et al., 2013; Di

Domenico et al., 2018; Heras-Sandoval et al., 2014; Mueed et al., 2018; Oddo, 2012). Hence, blocking 5-HT_6 receptor-mediated mTOR activation may have beneficial effects not only on cognition, but also on many pathological features of AD.

The critical influence of the mTOR pathway, under the control of 5-HT_6 receptors, in memory performance was recently confirmed in the context of dietary restriction (DR) in the mouse (Teng et al., 2019). As previously described in Chapter 3, mice subjected to DR show a reduced expression of the 5-HT_6 receptor and, consequently, a decrease in 5-HT_6 receptor-mediated mTOR signaling in the hippocampal tissue (Teng et al., 2019). In this study, the authors also showed that peripheral administration of WAY208466, a selective 5-HT_6 receptor agonist, abrogates DR-induced memory enhancement, whereas administration of SB399885, a 5-HT_6 receptor antagonist, does not further improve memory in DR mice. Furthermore, supplementation of food with phosphatidic acid, an mTORC1 activator, attenuates memory performance in DR mice and 5-$HT_6^{-/-}$ mice, while the mTORC1 inhibitor Everolimus mimics but does not further enhance the memory performance of DR and 5-$HT_6^{-/-}$ mice. Collectively, these results indicate that DR-induced reduction of 5-HT_6 receptor-operated mTOR signaling underlies the associated memory enhancement. The data also show a decreased complexity of dendrites and a reduction of dendritic length in hippocampal neurons of DR mice while the spine density was increased and LTP was enhanced, compared with mice fed *ad libitum*. Again, DR-associated structural alterations were caused by an attenuation of 5-HT_6 receptor-mediated mTORC1 signaling (Teng et al., 2019).

5.3. Role of the 5-HT_6 receptor interactome in epilepsy

In addition to its beneficial influence upon cognition, 5-HT_6 receptor blockade may be considered as a novel therapeutic strategy in epilepsy. Several studies have demonstrated that 5-HT_6 receptor antagonists have an anti-seizure activity by increasing the seizure threshold (Routledge et al., 2000; Stean et al., 2002; Wang et al., 2015). As previously mentioned (see Chapter 3), a higher expression of 5-HT_6 receptors was found in the cortex of TLE patients and in the pilocarpine rat model of temporal lobe epilepsy (Liu et al., 2019; Wang et al., 2015). Consequently, 5-HT_6 receptor-operated mTOR signaling as well as

Jab1/phospho-c-jun signaling were augmented in the brain of pilocarpine-treated rats (Liu et al., 2019; Wang et al., 2015). Corroborating numerous studies showing a role of the mTOR pathway in epileptogenesis (Bockaert and Marin, 2015), administration of a 5-HT_6 receptor antagonist before the induction of seizures increased seizure latency, decreased their severity (Wang et al., 2015) and relieved learning and memory deficits in pilocapine-treated rats (Liu et al., 2019).

6. Concluding remarks

This review illustrates the potential of deciphering the interactome of a GPCR to characterize novel mechanisms underlying the regulation of its functional status and to identify non-canonical signaling pathways involved in its physiological functions and associated pathologies. With regard to the 5-HT_6 receptor interactome, the functional outcome of only a minority of identified interactions has to date been characterized. Adding a further level of complexity, the receptor interactome might be dynamically regulated in time and space, depending on the receptor's conformational state and post-translational modifications. It is likely that many receptor interacting proteins successively participate in the highly temporally-regulated and complex processes under the control of 5-HT_6 receptors such as neuronal migration and differentiation, and cognition. Analysis of the interactome of native receptors, using for instance knock-in mice expressing epitope-tagged 5-HT_6 receptors, at different developmental stages or in pathological situations in which the receptor is implicated, will certainly provide important advance in the field.

Competing interest statement

The authors declare no competing interest with the present article.

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Figure legends

Figure 1. Subcellular localization of 5-HT_6 receptor in neurons. Double immunofluorescence staining of GFP (green) and the ciliary protein Arl13b (red) in primary cultured hippocampal neurons from 5-HT_6-GFP mouse embryos shows the presence of the 5-HT_6 receptor in the primary cilium where it is co-localized with Arl13b, and a more discrete expression in other neuronal compartments including the cell body, dendrites and spines. Original confocal images are illustrated. The white arrows point to primary cilia. Scale bar: 20 μm.

Figure 2. Pie chart representing the percentage of genes encoding proteins of the 5-HT_6 receptor interactome belonging to each indicated functional group. Percentage values and gene names are shown in Table 2. Functional categories overrepresented in the 5-HT_6 receptor interactome were assessed using the Cytoscape's ClueGo plugin (version 2.5.4) (Bindea et al., 2009). Genes were grouped according to their Biological Function Gene Ontology (GO), using all evidence except those Inferred from Electronic Annotation (IEA), and using the latest version of the Uniprot GOA database (06/26/2019). Functional groups were determined based on Kappa score level (≥ 0.3), as previously described (Bindea et al., 2009). For each overlapping group, only the most significant term is represented.

Figure 3. Regulation of 5-HT_6 receptor trafficking and signaling by interacting proteins. **A.** The association of the 5-HT_6 receptor with the Fyn kinase and MAP1B inhibits receptor internalization, thus enhancing receptor-operated signaling (Gs and Erk1,2 pathways). Association of the receptor with Fyn is essential for receptor-dependent Erk12 activation. Fyn can phosphorylate MAP1B but whether both proteins concomitantly interact with the receptor and whether they affect each other's interaction with the receptor remain to be established. **B.** The association of the 5-HT_6 receptor with Jab1 also inhibits receptor internalization. 5-HT_6 receptor activation leads to the disruption of the 5-HT_6 receptor-Jab1 complex and association of Jab1 with phosphorylated cJun. The Jab1-phospho-cJun complex is then

translocated to the nucleus where it enhances gene expression. **C.** The 5-HT_6 receptor interacts with the brain-enriched splicing regulator Nova-1. Nova-1 is translocated to the cytoplasm and associates with the 5-HT_6 receptor C-terminus when the receptor is over-expressed, thus reducing its splicing activity. Conversely, Nova-1 increases the internalization and degradation of the receptor by the proteasome. **D.** The association of the 5-HT_6 receptor with Sorting nexin 14 (SNX14) negatively regulates receptor-mediated signaling by i) promoting receptor internalization and degradation and ii) sequestrating Gαs. Phosphorylation of SNX14 by PKA induces the release of Gαs. The non-phosphorylated SNX14 is then translocated to the membrane where it binds to the i3 loop of the receptor. In **A-D.**, the receptor's partners are represented in blue and the dotted lines indicate the translocation of proteins.

Figure 4. Engagement of mTOR and Cdk5 signaling pathways by the 5-HT_6 receptor. *Left panel:* The 5-HT_6 receptor recruits mTORC1 and stimulates mTOR through a dual mechanism implicating the canonical PI3K/Akt/Tsc1,2/Rheb pathway and a physical interaction between 5-HT_6 receptor and mTOR. A non-physiological mTOR activation, under the control of 5-HT_6 receptors, compromises cognition in neuro-developmental models of schizophrenia and contributes to epileptogenesis. Correspondingly, blockade of 5-HT_6 receptor-elicited mTOR activation or food deprivation increases cognitive performances. 5-HT_6 receptor inhibition also increases seizure latency and decreases their severity in temporal lobe epilepsy. *Right panel:* The 5-HT_6 receptor interacts with Cdk5 and activates Cdk5 signaling in an agonist-independent manner *via* a mechanism involving receptor phosphorylation on Ser^{350} by Cdk5 bound to the receptor. The 5-HT_6 receptor also recruits and activates the non-receptor tyrosine kinase Fyn, which can contribute to 5-HT_6 receptor-operated Cdk5 activation through the induction of expression of the Cdk5 activator p35. Agonist-independent activation of Cdk5 by the 5-HT_6 receptor plays a key role in the migration of cortical neurons and the initiation of neurite growth.

Protein name	Uniprot ID	Gene name	Identification	Function	Reference
3-ketoacyl-CoA thiolase A	Q921H8	Acaa1a	Pull-down		Duhr et al.
3-mercaptopyruvate sulfurtransferase	Q99J99	Mpst	Pull-down		Duhr et al.
60kDa heat shock protein, mitochondrial	P63038	Hspd1	Co-IP (cilia)		Kohli et al.
Actin related protein 2/3 complex subunit 3	Q9JM76	Arpc3	Co-IP (cilia)		Kohli et al.
Actin related protein 2/3 complex subunit 4	P59999	Arpc4	Co-IP (cilia)		Kohli et al.
Actin related protein 2/3 complex subunit 5	Q9CPW4	Arpc5	Co-IP (cilia)		Kohli et al.
Actin related protein 2/3 complex subunit 5-like protein	Q9D898	Arpc5l	Co-IP (cilia)		Kohli et al.
Actin-Binding LIM protein 1	E9Q9C7	Ablim1	Co-IP (cilia)		Kohli et al.
Actin, aortic smooth muscle	P62737	Acta2	Co-IP (cilia)		Kohli et al.
Actin, cytoplasmic 2	P63260	Actg1	Co-IP (cilia)		Kohli et al.
Aldehyde dehydrogenase X	Q6UB35	Mthfd1l	Pull-down		Duhr et al.
Alpha-actinin 4	E9Q2W9	Actn4	Co-IP (cilia)		Kohli et al. Ha et al.
Ankycorbin	Q9EP71	Rai14	Co-IP (cilia)		Kohli et al.
Amphiphysin	Q7TQF7	Amph	Pull-down		Ha et al.
AP-2 complex subunit alpha-1	P17426	Ap2a1	Pull-down		Ha et al.

Protein name	Uniprot ID	Gene name	Identification	Function	Reference
AP-2 complex subunit alpha-2	P17427	Ap2a2	Pull-down		Ha et al.
AP-2 complex subunit beta	Q9DBG3	Ap2b1	Pull-down		Ha et al.
AP-3 complex subunit mu-2	Q8R2R9	Ap3m2	Pull-down		Duhr et al.
Apoptosis inducing factor 1	Q9Z0X1	Aifm1	Pull-down		Duhr et al.
Asparaginyl-tRNA synthetase	Q8BP47	Nars	Pull-down		Duhr et al.
Ataxin-10	P28658	Atxn10	Co-IP		Meffre et al.
Caldesmon 1	Q8VCQ8	Cald1	Co-IP (cilia)		Kohli et al.
Calumenin	Q6XLQ8	Calu	Co-IP (cilia)		Kohli et al.
cAMP dependant protein kinase type 1	Q9DBC7	Prkar1a	Pull-down		Duhr et al.
CDK5 regulatory subunit-associated protein 3	Q99LM2	Cdk5rap3	Co-IP		Meffre et al.
Clathrin coat assembly protein AP180	Q61548	Snap91	Pull-down		Ha et al.
Clathrin heavy chain 1	Q68FD5	Cltc	Co-IP		Meffre et al.
Coatomer subunit gamma-2	Q9QXK3	Copg2	Co-IP		Meffre et al.
Conserved oligomeric Golgi complex subunit 3	Q8CI04	Cog3	Co-IP		Meffre et al.
Coronin 1B	Q9WUM3	Coro1b	Co-IP (cilia)		Kohli et al.
Coronin 1C	Q9WUM4	Coro1c	Co-IP (cilia)		Kohli et al.

Protein name	Uniprot ID	Gene name	Identification	Function	Reference
Cullin-associated NEDD8-dissociated protein 2	Q6ZQ73	Cand2	Co-IP		Meffre et al.
Cyclin-dependent kinase 5	P49615	Cdk5	Co-IP, GST pull-down	Neuronal migration, neurite outgrowth, receptor constitutive activity	Meffre et al., Duhr et al.
Cytochrome b-c1 complex subunit 8	Q9CQ69	Uqcrq	Co-IP (cilia)		Kohli et al.
DCC-interacting protein 13-alpha	Q8K3H0	Appl1	Pull-down		Ha et al.
DnaJ protein homolog 2	P63037	Dnaja1	Pull-down		Duhr et al.
Dynamin-1	P39053	Dnm1	Pull-down		Ha et al.
Dynamin-1-like protein	Q8K1M6	Dnm1l	Pull-down		Ha et al.
Dynamin-2	P39054	Dnm2	Co-IP		Meffre et al. Ha et al.
Dynamin-3	Q8BZ98	Dnm3	Pull-down		Ha et al.
Dynamin-like 120kDa protein	P58281	Opa1	Pull-down		Duhr et al.
Elongation Factor Tu	Q8BFR5	Tufm	Pull-down		Duhr et al.
Ermin	Q5EBJ4	Ermn	Pull-down		Duhr et al.
Epsin-3	Q91W69	Epn3	Pull-down		Ha et al.
Exocyst complex component 2	Q9D4H1	Exoc2	Co-IP		Meffre et al.

Protein name	Uniprot ID	Gene name	Identification	Function	Reference
Exportin-4	Q9ESJ0	Xpo4	Co-IP		Meffre et al.
Exportin-T	Q9CRT8	Xpot	Co-IP		Meffre et al.
F-actin-capping protein subunit alpha-1	Q5RKN9	Capza1	Co-IP (cilia)		Kohli et al.
Flotilin 1	O08917	Flot1	Pull-down		Duhr et al.
Fyn kinase	P39688	Fyn	Y2H	Receptor trafficking, Erk signaling	Yun et al.
G alpha 11	P21278	Gna11	Pull-down		Duhr et al.
G protein Regulated Inducer of Neurite Outgrowth 1	Q3UNH4	Gprin1	Pull-down		Duhr et al.
Gamma glutamyl transferase 7	Q99JP7	Ggt7	Pull-down		Duhr et al.
Gelsolin	P13020	Gsn	Co-IP (cilia)		Kohli et al.
Heat shock protein HSP 90-beta	P11499	Hsp90ab1	Pull-down		Ha et al.
Importin-8	Q7TMY7	Ipo8	Co-IP		Meffre et al.
Imprinted and ancient gene protein (IMPACT)	O55091	Impact	Pull-down		Duhr et al.
JAB1(CSN5)	O35864	Cops5	Y2H	Receptor expression, c-jun signalling in the nucleus	Yun et al.
Kelch repeat and BTB domain protein	Q8BNW9	Kbtbd11	Pull-down		Duhr et al.
Leucine-rich repeat flightless interacting protein	E9QN52	Lrrfip2	Co-IP (cilia)		Kohli et al.

Protein name	Uniprot ID	Gene name	Identification	Function	Reference
LIM domain and actin-binding protein 1	Q9ERG0	Lima1	Co-IP (cilia)		Kohli et al.
Long-chain specific acyl-CoA dehydrogenase	P21174	dnaN	Pull-down		Duhr et al.
Lymphocyte-specific protein 1	A2A6J4	Lsp1	Co-IP (cilia)		Kohli et al.
Microtubule associated protein 1B	P14873	Map1b	Y2H	Receptor trafficking, modulation of receptor signaling	Kim et al.
Mitogen-activated protein kinase 1	P63085	Mapk1	Pull-down		Duhr et al.
Monofunctional C1-tetrahydrofolate synthase	Q3V3R1	Mthfd1l	Co-IP		Meffre et al.
Myosin Light Chain 12A	Q6ZWQ9	Myl12a	Co-IP (cilia)		Kohli et al.
Myosin Light Chain 6B	Q8CI43	Myl6b	Co-IP (cilia)		Kohli et al.
Myosin phosphatase rho-interacting protein	P97434	Mprip	Co-IP (cilia)		Kohli et al.
Myosin regulatory light polypeptide 9	Q9CQ19	Myl9	Co-IP (cilia)		Kohli et al.
NADP-dependant malic enzyme	Q8BMF3	Me3	Pull-down		Duhr et al.
Neurabin-2	Q6R891	Ppp1r9b	Co-IP (cilia)		Kohli et al.
Neurochondrin	Q9Z0E0	Ncdn	Co-IP		Meffre et al.
Neurofibromin	Q04690	Nf1	Co-IP	Receptor constitutive activity	Meffre et al. Deraredj-Nadim et al.

Protein name	Uniprot ID	Gene name	Identification	Function	Reference
Nexilin	Q7TPW1	Nexn	Co-IP (cilia)		Kohli et al.
Obg-like ATPase 1	Q9CZ30	Ola1	Pull-down		Duhr et al.
Paralemmin	Q9Z0P4	Palm	Pull-down		Duhr et al.
pericentriolar material 1 protein	Q9R0L6	Pcm1	Co-IP (cilia)		Kohli et al.
Phosphatidylinositol 3-kinase catalytic subunit type 3 (vps34)	Q6PF93	Pik3c3	Co-IP		Meffre et al.
Phosphomannomutase 1	O35621	Pmm1	Pull-down		Duhr et al.
Phostensin	Q8BQ30	Ppp1r18	Co-IP (cilia)		Kohli et al.
Poly(Rc)-binding protein 1	P60335	Pcbp1	Pull-down		Duhr et al.
Probable ATP-dependent RNA helicase DDX46	F8WHR6	Ddx46	Co-IP (cilia)		Kohli et al.
Protein flightless 1 homolog	Q9JJ28	Flii	Co-IP (cilia)		Kohli et al.
Protein phosphatase 1 regulatory subunit 1	Q9DBR7	Ppp1r12a	Co-IP (cilia)		Kohli et al.
Protein phosphatase 1A/PP2C alpha	P49443	Ppm1a	Pull-down		Duhr et al.
Protein phosphatase PTC7 homolog/TA-PP2C	Q6NVE9	Pptc7	Pull-down		Duhr et al.
Protein prune homolog	Q8BIW1	Prune1	Pull-down		Duhr et al.
Protein Rheb	Q540E6	Rheb	Pull-down	mTOR signalling	Duhr et al. Meffre et al.

Protein name	Uniprot ID	Gene name	Identification	Function	Reference
Protein-arginine deiminase type-2	Q08642	Padi2	Pull-down		Duhr et al.
Putative adenosylhomocysteinase 2	Q80SW1	Ahcyl1	Pull-down		Duhr et al.
Ragulator complex protein LAMTOR1	Q9CQ22	Lamtor1	Co-IP (cilia)		Kohli et al.
Ran-binding protein 6	Q8BIV3	Ranbp6	Co-IP		Meffre et al.
Regulatory-associated protein of mTOR	Q8K4Q0	Rptor	Co-IP		Meffre et al.
Reticulocalbin-2	Q8BP92	Rcn2	Pull-down		Duhr et al.
RNA Binding Protein Nova-1	Q9JKN6	Nova1	Y2H	Splicing activity of Nova-1, receptor expression	Kim et al.
Serine protein kinase ATM	Q62388	Atm	Co-IP		Meffre et al.
Serine/threonine-protein kinase ATR	Q9JKK8	Atr	Co-IP		Meffre et al.
Serine/threonine-protein kinase mTOR	Q9JLN9	Mtor	Co-IP	Cognition	Meffre et al.
Serine/threonine-protein kinase SMG1	Q8BKX6	Smg1	Co-IP		Meffre et al.
Serine/threonine-protein phosphatase 2A	Q67MZ3	STH1965	Pull-down		Duhr et al.
Serine/threonine-protein phosphatase 2A 56kDa regulatory subunit epsilon isoform	Q61151	Ppp2r5e	Co-IP (cilia)		Kohli et al.
Serine/threonine-protein phosphatase PP1-beta catalytic subunit	P62141	Ppp1cb	Co-IP (cilia)		Kohli et al.

Protein name	Uniprot ID	Gene name	Identification	Function	Reference
Sorting-Nexin-14	Q8BHY8	Snx14	Pull-down	Receptor trafficking and signal transduction	Ha et al.
Src substrate cortactin	Q60598	Cttn	Co-IP (cilia)		Kohli et al.
Succinate dehydrogenase (ubiquinone) Flavoprotein subunit, mitochondrial	Q8K2B3	Sdha	Co-IP (cilia)		Kohli et al.
Supervillin	Q8K4L3	Svil	Co-IP (cilia)		Kohli et al.
Synaptic vesicle membrane protein 1	Q62465	Vat1	Pull-down		Duhr et al.
TELO2-interacting protein 1 homolog	Q91V83	Tti1	Co-IP		Meffre et al.
Telomere length regulation protein TEL2 homolog	Q9DC40	Telo2	Co-IP		Meffre et al.
Thioredoxin-like protein 1	Q8CDN6	Txnl1	Pull-down		Duhr et al.
Transportin-1	Q8BFY9	Tnpo1	Co-IP		Meffre et al.
Tropomodulin-3	Q9JHJ0	Tmod3	Co-IP (cilia)		Kohli et al.
Tropomyosin 3 related sequence 7	D3Z2H9	Tpm3-rs7	Co-IP (cilia)		Kohli et al.
Tropomyosin alpha-1 chain	P58771	Tpm1	Co-IP (cilia)		Kohli et al.
Tropomyosin alpha-3 chain	E9Q7Q3	Tpm3	Co-IP (cilia)		Kohli et al.
Tropomyosin alpha-4 chain	Q6IRU2	Tpm4	Co-IP (cilia)		Kohli et al.
Tropomyosin beta chain	P58774	Tpm2	Co-IP (cilia)		Kohli et al.

Protein name	Uniprot ID	Gene name	Identification	Function	Reference
Tubulin-specific chaperone D	Q8BYA0	Tbcd	Co-IP		Meffre et al.
Unconventional myosin-VI	E9Q175	Myo6	Co-IP (cilia)		Kohli et al.
Vacuolar protein sorting-associated protein 35	Q9EQH3	Vps35	Pull-down		Ha et al.
Vesicle sortin protein 26B	Q8C0E2	Vps26b	Pull-down		Duhr et al.
Vesicle-fusing ATPase	P46460	Nsf	Co-IP		Meffre et al.
WASP family protein number 1	Q8R5H6	Wasf1	Pull-down		Duhr et al.
WD repeat-containing protein 1	O88342	Wdr1	Co-IP (cilia)		Kohli et al.

Table 1: List of 5-HT_6 receptor interacting proteins identified. For each protein, the Uniprot ID, gene name, method of purification, functional impact of its interaction with 5-HT_6 receptor (whenever available) and reference are indicated.

GO	GO term	Gene number	% of genes	Gene names
GO:0007212	dopamine receptor signalling pathway	3	2.4	Gna11, Palm, Vps35
GO:0001919	regulation of receptor recycling	2	1.6	Lamtor1, Nsf
GO:0010762	regulation of fibroblast migration	3	2.4	Acta2, Appl1, Coro1c
GO:0016601	Rac protein signal transduction	3	2.4	Dnm2, Nf1, Wasf1
GO:0030049	muscle filament sliding	2	1.6	Myl6b, Tpm1
GO:0030239	myofibril assembly	5	4	Actg1, Dnm1l, Prkar1a, Tmod3, Wdr1
GO:0046847	filopodium assembly	3	2.4	Dnm3, Palm, Ppp1r9b
GO:0031111	negative regulation of microtubule polymerization or depolymerization	4	3.2	Map1b, Wasf1, Tbcd, Prune1
GO:0043666	regulation of phosphoprotein phosphatase activity	7	5.6	Hsp90ab1, Mprip, Ppp1r12a, Ppp1r9b, Ppp2r5e, Wdr1, Mtor
GO:0046822	regulation of nucleocytoplasmic transport	7	5.6	Cdk5, Mapk1, Nf1, Ppm1a, Ppp1r12a, Xpo4, Map1b
GO:2000331	regulation of terminal button organization	5	4	Dnm1l, Vps35, Snap91, Wdr1, Dnm2
GO:0030036	actin cytoskeleton organization	46	36.8	Actg1, Actn4, Arpc3, Arpc4, Arpc5, Arpc5l, Atr, Cald1, Capza1, Cdk5, Coro1b, Coro1c, Cttn, Dnaja1, Dnm1l, Ermn, Flii, Gsn, Lima1, Mtor, Nf1, Ppp1r9b, Prkar1a, Tmod3, Tpm1, Tpm2, Tpm3, Tpm3-rs7, Tpm4, Wasf1, Wdr1, Dnm2, Hsp90ab1, Map1b, Rptor, Nsf, Snx14, Atm, Impact, Ppm1a, Prune1, Snap91, Tbcd, Vps35, Ablim, Opa1
GO:1903426	regulation of reactive oxygen species biosynthetic process	9	7.2	Dnm2, Fyn, Hsp90ab1, Hspd1, Mtor, Coro1b, Lsp1, Cdk5, Lrrfip2
GO:0051646	mitochondrion localization	10	8	Dnm1l, Map1b, Opa1, Wasf1, Wdr1, Ap3m2, Copg2, Cttn,

GO	GO term	Gene number	% of genes	Gene names
				Snap91, Tbcd
GO:0030100	regulation of endocytosis	17	13.6	Actn4, Ap2a1, Appl1, Dnm1, Dnm1l, Dnm2, Flot1, Nsf, Snap91, Wdr1, Coro1c, Nf1, Tbcd, Palm, Coro1b ,Mtor, Cdk5
GO:0006898	receptor-mediated endocytosis	30	24	Ap2a1, Ap2a2, Ap2b1, Cltc, Cttn, Dnm1, Dnm1l, Dnm2, Dnm3, Flot1, Myo6, Nsf, Snap91, Wasf1, Wdr1, Actg1, Cdk5, Fyn, Opa1, Rheb, Vps35, Actn4, Ap3m2, Snap91, lamtor1, Appl1, Amph, Palm, Mtor, Ppp1cb
GO:0097581	lamellipodium organization	21	16.8	Ablim1, Arpc5, Coro1b, Coro1c, Cttn, Dnm2, Mtor, Wasf1, Cdk5, Mapk1, Ppp1r9b, Fyn, Hsp90ab1, Hspd1, Lamtor, Rptor, Mthfd1l, Ablim1, Impact, Actn4, Dnaja1
GO:0031929	TOR signaling	32	25.6	Atm, Lamtor1, Mtor, Rheb, Rptor, Telo2, Tti1, Ppp1r9b, Cdk5, Dnm2, Mapk1, Fyn, Hspd1, Lamtor, Ap2a1, Cltc, Ppp1cb, Dnm1l, Opa1, Nf1, Wdr1, Mprip, Dnaja1, Dnm3, Aifm1, Cdk5rap3, Pik3c3, Atr, Smg1, Hsp90ab1, Myo6, Gsn
GO:0099173	postsynapse organization	37	29.6	Cdk5, Cttn, Dnm1l, Dnm3, Fyn, Opa1, Vps35, Mtor, Ppp1r9b, Dnm2, Mapk1, Map1b, Nf1, Coro1c, Wdr1, Coro1b, Palm, Rheb, Aifm1, Atm, Hsp90ab1, Hspd1, lamtor, Rptor, Ppm1a, Ppp1r12a, Xpo4, Dnaja1, Actg1, Wasf1, Vat1, Ap3m2, Copg2, Myo6, Snap91, Pptc7, Dnaja1

Table 2: Genes encoding proteins of the 5-HT_6 receptor interactome retrieved in overrepresented GO categories. For each GO category, the number of genes, the % of genes (total number of unique proteins identified in the 5-HT_6 receptor interactome: 125) and the names of the genes corresponding to the GO term are indicated.

Figure(s)

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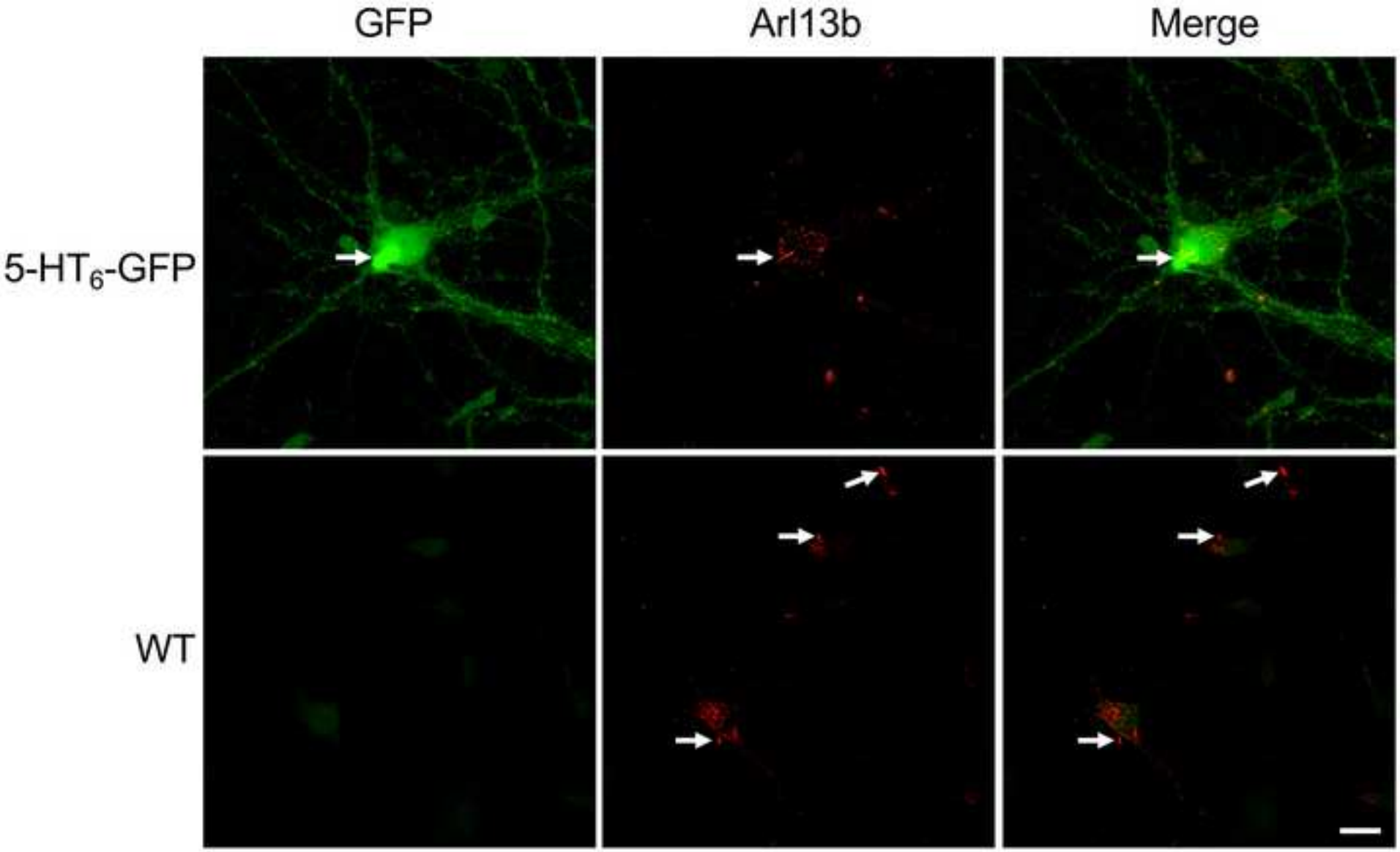

Figure 1

Figure(s)

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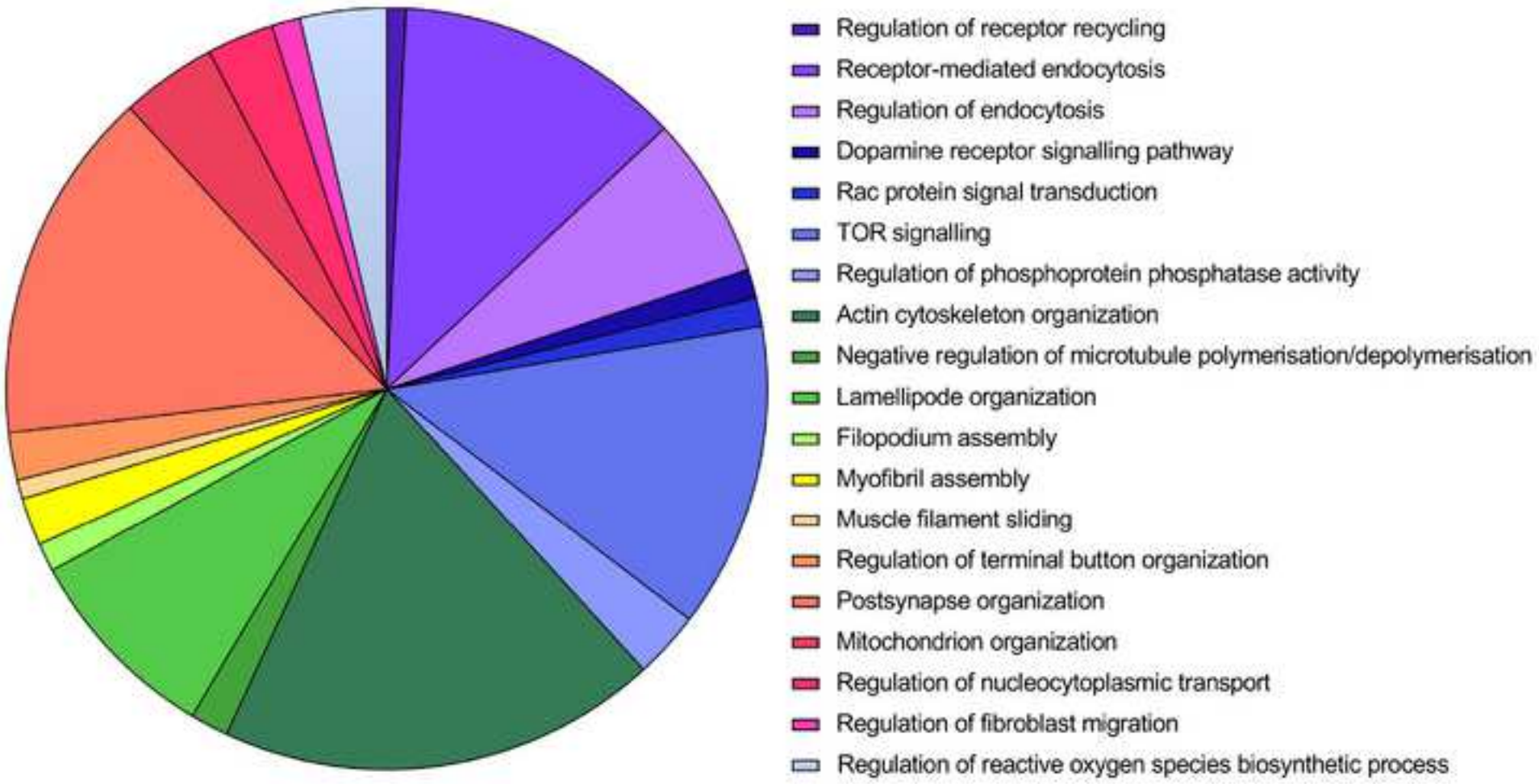

Figure 2

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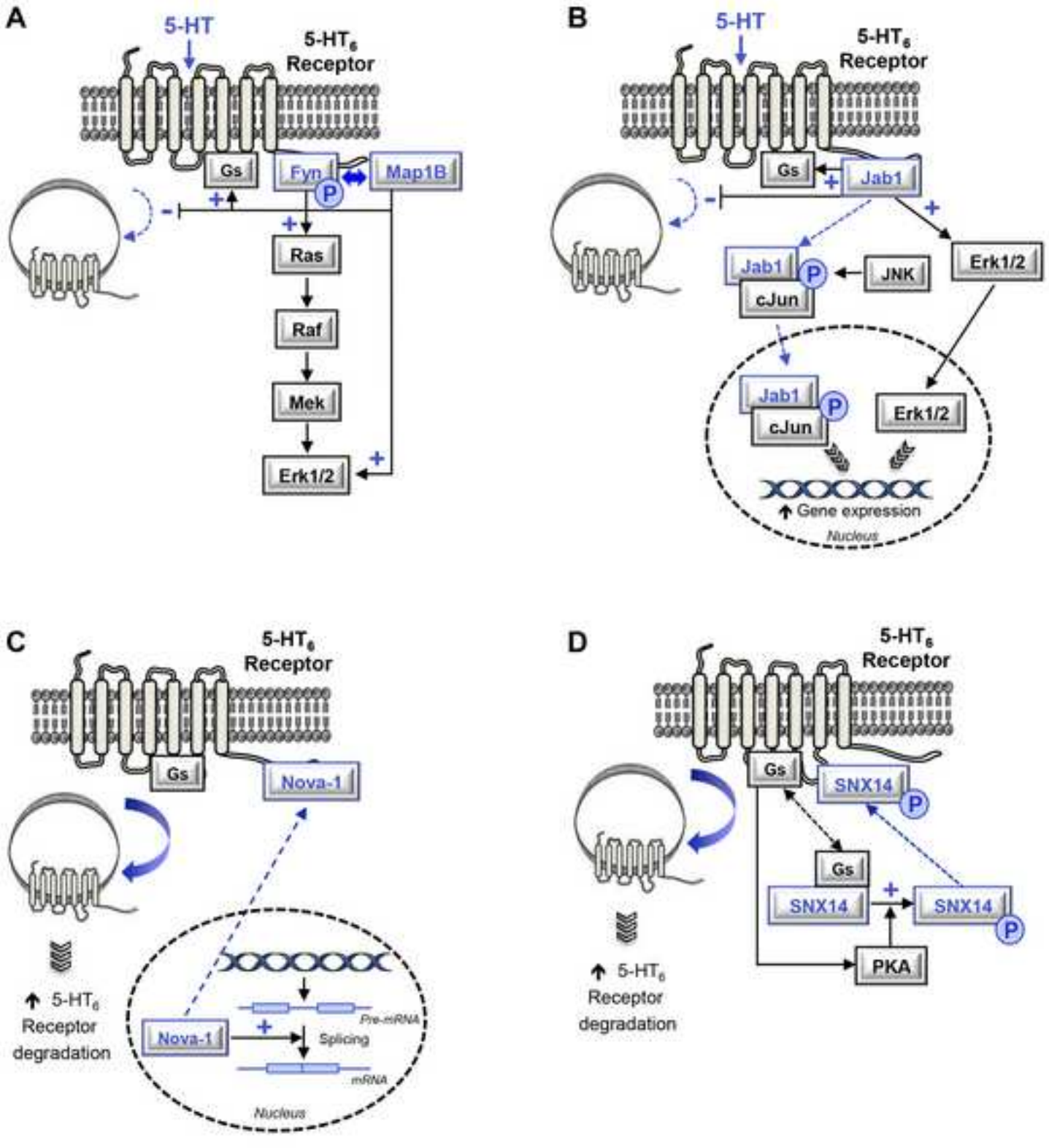

Figure 3

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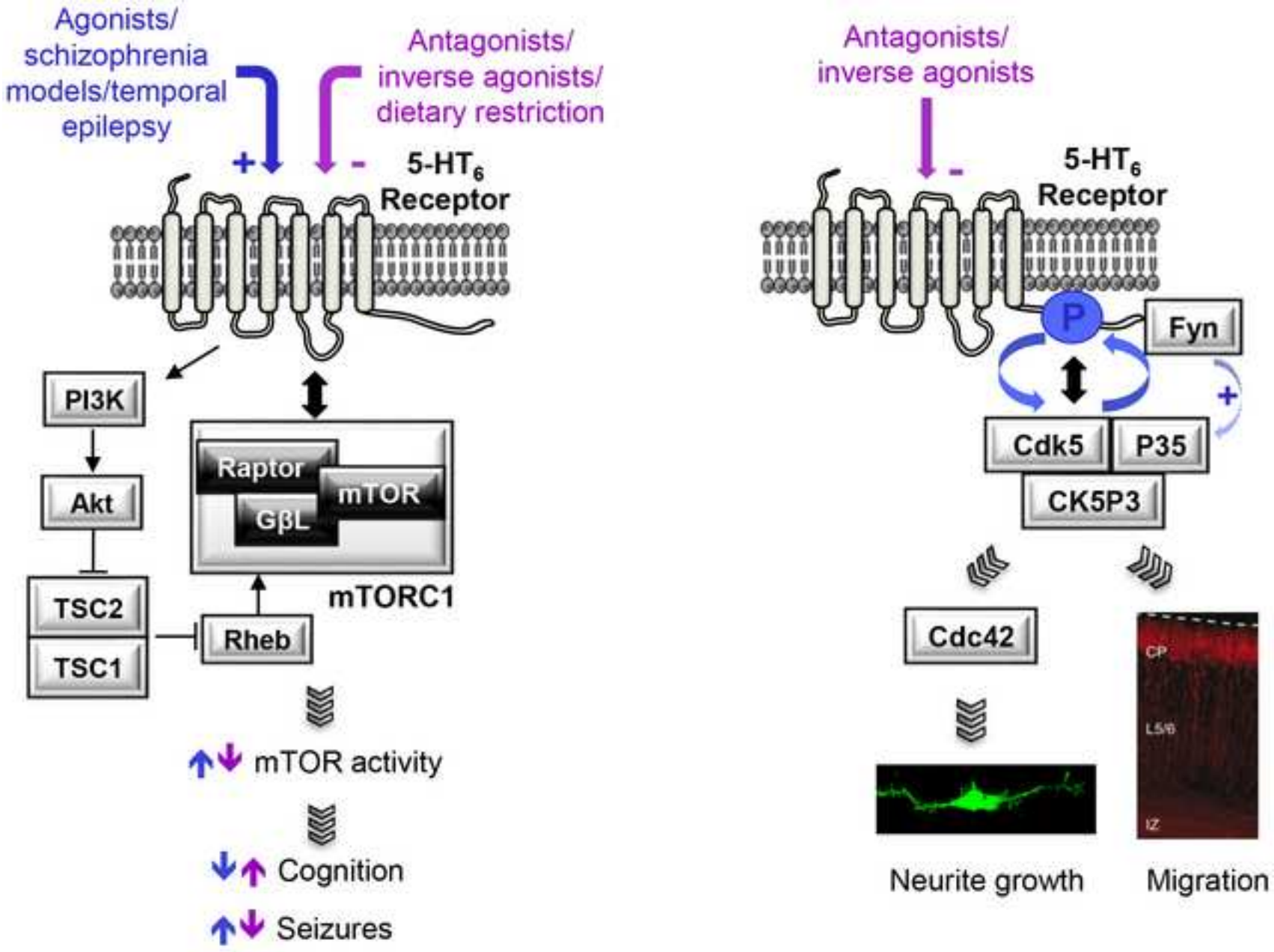

Figure 4