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5-HT₄ receptors, a place in the sun: Act two

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Abstract

5-HT₄ receptors control brain physiological functions such as learning and memory, feeding and mood behaviour as well as gastro-intestinal transit. 5-HT₄ receptors are one of the 5-HT receptors for which the available drugs and signalling knowledge are the most advanced. Several therapeutic 5-HT₄ receptor drugs have been commercialized. Therefore, the hope that 5-HT₄ receptors could also be the target for brain diseases is reasonable. Several major devastating illnesses could benefit from 5-HT₄ receptors-directed therapy such as Alzheimer's disease, feeding-associated diseases such as anorexia and major depressive disorders.

Introduction

G protein-coupled receptors (GPCRs) are conserved molecules which have been central, during evolution to recognize and transduce external (e.g. odours, gustative molecules, light) as well as cell-cell communication signals (hormones, neurotransmitters, some growth factors) [1]. They comprise 2% of the human genome and remain central for the understanding of human physiology and human diseases. 40-50% of all marketed drugs act directly to modulate GPCRs even if less than 20% of GPCRs (non-odorant receptors) are currently targeted. 5-HT₄ receptors reached their "place in the sun" of GPCR pharmacology almost 20 years ago [2*] and remain the matter of intense research especially in brain and gastro-intestinal systems [3*]. Indeed, the hope that 5-HT₄ receptor agonists could be successful drugs in gastro-intestinal pathologies and in particular for chronic idiopathic constipation is high. Several compounds have already been tested [3*]. Some have been withdrawn for adverse but non 5-HT₄ receptor-mediated side effects. For example, cisapride is known to inhibit hERG channels [4]. However, prucalopride, has just been commercialized [5]. In brain, none 5-HT₄ receptor ligand has yet reached such a development level, even if a clinical trial for Alzheimer's disease reached phase IIb [6,7]. In addition to this pathology, important discoveries indicate that two others mental diseases, such as depression and feeding disorders, could benefit from modulation of 5-HT₄ receptor signalling [8*-11*]. Recently, it has been proposed that GPCR interacting proteins (GIPs) may be used for modulating GPCR-signalling in pathologies [12*]. For example, p11, a GIP that modulates the trafficking of 5-HT_{1B} and 5-HT₄ receptors to the plasma membrane, has been expressed, in nucleus accumbens (NAc) to reverse depression like behaviours in mice [13**]. In this short review, we will only discuss recent studies on new aspects of 5-HT₄ receptor signalling including non G protein-mediated events [14*], the nature and functions of the 5-HT₄ receptor-associated GIPs [12*] as well as new evidences for a role of those receptors in important aforementioned brain diseases. Is a "place in the sun" for 5-HT₄ receptors, in brain diseases treatments close to be reached?

New vistas in 5-HT₄ receptors signalling

The capacity of 5-HT₄ receptors to couple and initiate signalling pathways through multiple G proteins, especially G_s, G₁₃ and in some cell lines G_q and G_i, has already been documented in precedent reviews [3*,15]. Here, we are highlighting recent studies which provide evidence that 5-HT₄ receptor stimulation mediates G protein-independent pathways, the first one being ERK activation through phosphorylation and activation of the 5-HT₄ receptor-associated non-receptor tyrosine kinase Src (Figure 1). 5-HT₄ receptor-mediated ERK activation is the first example that involves neither a classical G protein signalling (G_s/G_q/G_i) and associated messengers, nor β -arrestins [14*]. This result does not seem to be cell specific, because 5-HT₄ receptors stimulation activates Src in HEK293, neurons [14*] and also in epithelial cell lines. Indeed, in the enterocyte cell line Caco 2, 5-HT₄ receptor-induced Src activation was required to activate the PLC γ ₁ pathway and to inhibit Na⁺/H⁺ exchanger [16] (Figure 1).

As nothing was known about desensitization of GPCRs acting in a G protein-independent manner, we investigated this question using 5-HT₄ receptors. We reported that the 5-HT₄ receptor-operated Src/ERK pathway, but not the G_s pathway, was negatively regulated by GPCR kinase 5 (GRK5) physically pre-associated with the proximal C-terminal region of the receptor in both human HEK293 cells and mouse colliculi neurons [17**]. This inhibition required two sequences of events: the association of β -arrestin1 to a phosphorylated serine/threonine cluster located within the receptor C-terminal domain and the phosphorylation, by GRK5, of β -arrestin1 (at Ser412) bound to the receptor. Phosphorylated β -arrestin1, in turn, prevented activation of Src constitutively bound to 5-HT₄ receptors, a necessary step in receptor-stimulated ERK signalling [17**] (Figure 1).

GPCR interacting proteins (GIPs) associated with 5-HT₄ receptors

GPCR functions are controlled by numerous protein partners named GIPs [12*]. These interactions create additional opportunities for G protein-dependent but also independent signalling. In a first study using a proteomic approach, we found ten proteins that have been identified to interact directly with the C-terminus of 5-HT_{4(a)} receptor variant and three that associate with the C-terminus of 5-HT_{4(e)} receptor variant, most through PDZ domains. Among the more interesting interacting proteins are the Na⁺/H⁺ exchanger regulatory factor (NHERF) and sorting nexin 27 (SNX27) [18*]. The group of P. Greengard recently documented the interaction of 5-HT₄ receptors with p11. p11 (also called S100A10 or calpactin-1 light chain or annexin-2 light chain) is an inducible 97-amino acid protein member of the S100 superfamily comprising 10-14 kDa, EF hand-containing Ca²⁺-binding proteins, which transmit Ca²⁺-dependent cell-regulatory signals. This protein is present in a large number of organisms, interact with trafficking proteins, several channels but also with the 5-HT_{1B} and the 5-HT₄ receptors [19**]. Interaction of both receptors with p11 increased their cell surface localization and, correspondingly, enhanced their associated signal transduction [20*].

The majority of GIPs associated to 5-HT receptors have so far been isolated by *in vitro* binding assays using GPCR C-termini as baits [18*,21,22]. Recently, we identified more than 150 new protein partners of the 5-HT₄ receptor by a shot gun proteomic approach associating immunoprecipitation of the N-terminally tagged receptor, expressed in an heterologous system (HEK293 cells), and systematic identification using tandem mass spectrometry. Several of the identified proteins are known to act on the trafficking and/or the metabolism of APP (amyloid precursor protein) (unpublished data), reinforcing the proposal that 5-HT₄ receptors could be a target for Alzheimer disease [23*].

Learning and memory and Alzheimer's disease

Pro-cognitive effects of 5-HT₄ receptor agonists have been described, both on short-term memory (such as social olfactory memory) or long-term olfactory memory (such as olfactory associative memory, for a review see [6]). 5-HT₄ receptor-induced increase in memory acquisition has been seen in autoshaping task, object recognition, Morris water-maze, task with long inter-trial intervals (2 h), delayed matching performance and impeded spontaneous alteration scores [6]. Such positive effects on learning and memory could rely on a 5-HT₄ receptor-mediated LTP (long term potentiation) induction that has been documented in the amygdala [24*] and suggested in the hippocampus [25], where 5-HT₄ receptor activation cause a long-lasting excitability increase in CA1 pyramidal neurons [26]. The complexity of 5-HT₄ receptor effects on the synaptic plasticity of the Shaffer-collateral/CA1 hippocampal synapses in correlation with the type of learning has been highlighted [25]. The conclusion being that 5-HT₄ receptor activation may be beneficial for particular types of information acquisition that depend on LTP such as exploration of an empty novel environment and detrimental for other types of learning which required LTD (long term depression) such as exploration of a new environment containing unfamiliar objects. A recent report shows that an increase in cAMP phosphodiesterases (PDEs) activity occurs in the prefrontal cortex (PDE4) and hippocampus, following 5-HT₄ receptor stimulation. This may be part of a negative feed-back regulation mediated by cAMP [27]. Moreover, we contributed to show that the promnesic effect of 5-HT₄ receptor activation is also mediated by a potentiation of learning-induced spine growth in the hippocampus [28**]. In 5-HT₄ receptor knock-out mice, the loss of function mutation on learning and memory appears to be circumvented by adaptative changes in cholinergic systems [29], supporting the fact that 5-HT₄ receptor can have a role to play in Alzheimer's disease

(AD). Indeed, 5-HT₄ receptors have been reported to promote non-amyloidogenic processing of the amyloid precursor protein (APP) by stimulating the α -secretase [23*,30]. Along these lines, a selective 5-HT₄ receptor partial agonist, RS 67333 inhibits the generation of the β -amyloid peptide (A β) in primary cortical neurons [30]. Interestingly, two 5-HT₄ receptor partial agonists, the SL 65.0155 from Sanofi-Aventis [7] and VRX-03011 (Epix Pharmaceuticals [31]) have reached clinical phase II for the treatment of Alzheimer's disease. Both compounds act synergically with cholinesterase inhibitors on rodent memory. All these studies correlate with reports documenting a significant decrease in the density of 5-HT₄ receptor binding sites in cortical and hippocampal regions in the Alzheimer brain [32-34].

5-HT₄ receptors for treating feeding disorders

The first evidence that 5-HT₄ receptors could be implicated in feeding disorders was obtained using the 5-HT₄ receptor knock-out mice [11*]. Indeed, the defective mutation of the *mHtr4* gene encoding 5-HT₄ receptors reduced hypophagia following restraint stress, a possible animal model for *anorexia nervosa* [35]. It has been proposed that motivation disorders whereby patients self-impose food refusal despite an energy request (anorexia-like behaviour) could be seen as prominent disturbances in a reward structure (nucleus accumbens: NAc) rather than in the hypothalamus [10**,36]. We have recently described that the rewarding drug MDMA (3,4-*N*-methylenedioxymethamphetamine) reduces feeding via 5-HT₄ receptor activation and their downstream signalling cascade cAMP/PKA synthesis of CART, an anorectic and addiction peptide (CART: Cocaine- and amphetamine-regulated transcript) [10**]. Intra-accumbal injection of a 5-HT₄ receptor antagonist (RS 39604) or siRNA-mediated 5-HT₄ receptors knock-down decrease satiety [10**,34] and indicate a 5-HT₄ receptors localization on intrinsic, likely GABAergic neurons of the NAc (Figure 2). The neuronal network of addiction may thus include the neuronal pathway related to anorexia [10**]. Full 5-HT₄ receptor antagonists may thus be useful drugs to treat anorexia related disorders. It is interesting to note that oral administration of the 5-HT₄ receptor partial agonist, mosapride citrate, increased feeding in an animal model of obesity (*ob/ob*) mice [37] (mosapride being a partial agonist, can also partially inhibit 5-HT₄ receptors activation by endogenous 5-HT). Current studies of the involvement of 5-HT₄ receptors and related mechanisms in stress-induced hypophagia and in addiction to drugs of abuse (*e. g.* cocaine) are in progress. Studies conducted in mice have found a promising opening in human therapy. Indeed, recently, the density of 5-HT₄ receptors was found to be decreased in Alzheimer's disease patients also suffering from hyperphagia [34]. New development of PET radioligand such as [¹¹C]SB207145 should facilitate *in vivo* brain imaging of 5-HT₄ receptors in human brain disorders [38].

Treating depression

Few years ago, polymorphisms of the *Htr4* gene encoding the 5-HT₄ receptors have been highly correlated with major depression and/or bipolar disorders and *post-mortem* examination of brain of depressed suicide victims indicate an altered expression of 5-HT₄ receptors (for a review see [8*]). However, the evidence that 5-HT₄ receptors may be a direct target for treating depression is more recent [8*]. It is well known that inhibitors of 5-HT and nor-epinephrine reuptake are central in the therapeutic action of antidepressants. In particular, 5-HT reuptake inhibitors (SSRI) are commonly used. The final effect of these drugs seems to be an increase in the tonic 5-HT release and activation of post-synaptic 5-HT receptors, in particular 5-HT_{1A} receptors in limbic structures such as hippocampus. However, this effect is delayed and obtained only after 3-8 weeks. One of the possible explanations may be that this delay is due to

the period needed to obtain desensitization of the self-inhibition of 5-HT neurons by somatodendritic 5-HT_{1A} receptors. At the beginning of SSRI treatment, 5-HT neuron activities are quasi suppressed and symptoms may be increased, in particular anxiety. Thus, this period is problematic and drugs rapidly activating 5-HT neurons may facilitate SSRI treatments and even be antidepressant by themselves. 5-HT₄ receptors were first shown to increase 5-HT release in the hippocampus [39] and then to be a tonic activator of about half of dorsal raphe nucleus (DRN) 5-HT neurons called "responders" neurons. It appears that those neurons have a higher basal firing rate (1.9Hz) than non-responders ones (1.3 Hz) [40]. Thus, i.p. administration of the 5-HT₄ receptor antagonists (such as GR 125487) highly reduced the basal firing rate of "responders" but were inactive on "non-responders" [40]. Conversely, "responders" but not "non-responders" were activated by i.p. administration of 5-HT₄ receptor agonists such as cisapride, prucalopride or RS 67333 indicating that 5-HT₄ receptors have also a phasic control of firing [8*,40]. More interestingly, the inhibitory effect of SSRI on 5-HT neuron firing was counteracted by prucalopride in neurons discharging at 2Hz or higher but not when the frequency was < 1Hz [8*]. When an SSRI such as citalopram was co-administrated with prucalopride or RS 67333 for 3 days, the activity of DRN 5-HT neurons was stimulated instead of inhibited when citalopram was given alone [9**]. These results are in accordance with the observation that the average firing rate of DRN 5-HT neurons was reduced by more than 50% in 5-HT₄ receptor knock-out mice compared to WT [41]. Since the effect of 5-HT₄ receptor agonists on firing rate was observed after i.p. delivery but not after a direct delivery at the DR level, indicates that the 5-HT₄ receptors involved were not localized in DRN [42*] (Figure 2). Since 1) the electrical stimulation of medial prefrontal cortex (mPFC) stimulates the electrical activity of 40-45% of DRN 5-HT neurons, 2) the mPFC 5-HT_{1A}Rs mediated a "long-loop feedback" on about half of DRN 5-HT neurons and 3) the 5-HT₄ and 5-HT_{1A} receptors are co-localized on pyramidal mPFC, it was tempting to hypothesized that the 5-HT₄ receptor-dependent control of DRN neuronal activity was originating from mPFC. Indeed, pyramidal cells express 5-HT₄ receptors (see [8*,42*,43*] for details) (Figure 2). Viral-induced over-expression of 5-HT₄ receptors in the mPFC but not in others discrete brain areas induced a marked increase in DR 5-HT neuronal firing [42*]. In conclusion, those convergent results demonstrated that mPFC 5-HT₄ receptors exert an excitatory influence of 5-HT neurons that compensates (at least after 3 days of activation with a 5-HT₄ receptor agonist) the initial suppression of DR 5-HT neurons activity by SSRI [9**]. Thus, 5-HT₄ receptor agonists may be antidepressant by themselves or reduce the delay of action of SSRI. This has been clearly the case as shown by Lucas *et al.* [9**] on a series of biological and behavioural parameters in rats. The biological parameters were including neurogenesis of hippocampal granular cells (GCs), which are also expressing 5-HT₄ receptors (Figure 2) [9**]. Interestingly, Kobayashi *et al.* [44**] described recently an other effect of fluoxetine, a classical SSRI, on GCs. Fluoxetine induced GC "dematuration" which developed along with increases in efficacy of 5-HT₄ receptor-dependent neuromodulation of the GCs to CA3 synapses and was almost suppressed in mice lacking those receptors [44**]. The fact that chronic SSRI treatments led to 5-HT₄ receptor stimulation is also suggested by the observation that such treatments induced a down-regulation of those receptors in hippocampus (particularly CA1 region), striatum, and substantia nigra but, interestingly, not in mPFC [45,46]. Finally, the p11 saga has highlighted the connection between 5-HT₄ receptors and depression. This protein has been identified as a new antidepressant target. p11 is down-regulated in depressive-like state in rodents, in the HelplessH/Rouen mice, and human brain, and is up-regulated following anti-depressant treatments including electroconvulsive treatments [19**]. p11 knock-out mice display a depressive-like phenotype whereas mice over-expressing p11 behave as those treated with antidepressant [19**]. p11 interacts with both 5-HT_{1B} and 5-HT₄ receptors (but not with many others GPCRs), facilitates their cell surface localization as well as their signalling and neuronal

functions [19**]. Interestingly, 5-HT_{1B} receptors are pre-synaptically localized and their effects on the inhibition of cortico-accumbal glutamatergic transmission require p11 [19**]. This observation, as well as human functional imaging or results from therapeutic deep brain stimulation of depression corroborating animal studies suggesting that reduce activity in this region (see [13**]) can promote depression, led Alexander *et al.* [13**] to investigate the possibility that adeno-associated virus (AAV) p11 gene-transfer to NAc could reverse their depression like behaviours. This was indeed the case and was associated with an increase in 5-HT_{1B} receptors in density [13**]. The data did not indicate if this increase is taking place on NAc presynaptic (glutamatergic?) terminals or on the intrinsic NAc neurons or both. This will be important to know. Is the p11-mediated increase in 5-HT₄ receptor membrane targeting also involved in this gene therapy? The presence of 5-HT₄ receptors on intrinsic NAc neurons as well as their ability to increase cAMP and CREB phosphorylation may suggest such a role [10**]. However note that p11 is also controlling the trafficking of several ionic channels see [19**]. Is p11 gene-transfer in human treatment of severe depression, a science fiction story or a reasonable hope? The future will tell us.

Conclusion

Drug development is a long and a non-linear process with many go and no periods. Several 5-HT₄ receptor drugs have been on the market for gastro-intestinal health problems such as gastro-oesophageal reflux and irritable bowel syndrome associated with constipation and recently constipation in women in whom laxatives were inefficient. This experience should be beneficial for development of brain 5-HT₄ receptor acting drugs. Here again, the process is very slow. Some have been tested in clinic for Alzheimer's disease but the processes were stopped either for lack of significant efficacy in phase IIb (SL 65.0155 from Sanofi-Aventis) or for economical reasons (VRX-03011 from Epix Pharmaceuticals). However, the clinical trials for Alzheimer's disease are problematic due to heterogeneity of patients and lack of biomarkers. More work is needed on the role of 5-HT₄ receptors on cellular biology of APP. We have recently performed a deep proteomic analysis of 5-HT₄ receptors "receptosome" and found that it includes several important proteins known to be implicated in the trafficking and metabolism of APP (in preparation). The hope is also that 5-HT₄ receptors will reach clinical evaluation for feeding-associated diseases such as anorexia, the third chronic illness of adolescent in industrialized countries and major depressive disorders identified as the leading cause of disability in the Western World for those aged 15 to 44 years.

References

1. Bockaert J, Pin JP: **Molecular tinkering of G protein-coupled receptors: an evolutionary success.** *EMBO J* 1999, **18**:1723-1729.
- *2. Bockaert J, Fozard JR, Dumuis A, Clarke DE: **The 5-HT₄ receptor: a place in the sun.** *Trends Pharmacol Sci* 1992, **13**:141-145.
A review giving the pharmacological and functional arguments in favor of the existence of a fourth 5-HT receptor in addition to the 5-HT_{1,2,3} receptors known at that time.
- *3. Bockaert J, Claeysen S, Compan V, Dumuis A: **5-HT(4) receptors: history, molecular pharmacology and brain functions.** *Neuropharmacology* 2008, **55**:922-931.
Review on 5-HT₄ receptors based on the I.H. Page's lecture given at the Serotonin club in Oxford, 2008.
4. Kamiya K, Niwa R, Morishima M, Honjo H, Sanguinetti MC: **Molecular determinants of hERG channel block by terfenadine and cisapride.** *J Pharmacol Sci* 2008, **108**:301-307.
5. Manabe N, Wong BS, Camilleri M: **New-generation 5-HT₄ receptor agonists: potential for treatment of gastrointestinal motility disorders.** *Expert Opin Investig Drugs* 2010, **19**:765-775.
6. Bockaert J, Claeysen S, Compan V, Dumuis A: **5-HT₄ receptors.** *Curr Drug Targets CNS Neurol Disord* 2004, **3**:39-51.
7. Moser PC, Bergis OE, Jegham S, Lothead A, Duconseille E, Terranova JP, Caille D, Berque-Bestel I, Lezoualc'h F, Fischmeister R, et al.: **SL65.0155, a novel 5-hydroxytryptamine(4) receptor partial agonist with potent cognition-enhancing properties.** *J Pharmacol Exp Ther* 2002, **302**:731-741.
- *8. Lucas G: **Serotonin receptors, type 4: a new hope?** *Curr Drug Targets* 2009, **10**:1085-1095.
A comprehensive review on the potential use of 5-HT₄ receptors as a target for treating depression
- **9. Lucas G, Rymar VV, Du J, Mnie-Filali O, Bisgaard C, Manta S, Lambas-Senas L, Wiborg O, Haddjeri N, Pineyro G, et al.: **Serotonin(4) (5-HT(4)) receptor agonists are putative antidepressants with a rapid onset of action.** *Neuron* 2007, **55**:712-725.
This report is giving compelling and very important evidence, from preclinical animal models, that agonists acting at the 5-HT₄ receptors be fast-acting antidepressants and may act in synergy with classical antidepressants.
- **10. Jean A, Conductier G, Manrique C, Bouras C, Berta P, Hen R, Charnay Y, Bockaert J, Compan V: **Anorexia induced by activation of serotonin 5-HT₄ receptors is mediated by increases in CART in the nucleus accumbens.** *Proc Natl Acad Sci U S A* 2007, **104**:16335-16340.
Although 5-HT receptors, in the hypothalamus, has been central for the 5-HT-mediated hypophagia, this article describes an important role of 5-HT₄ receptors expressed in the Nucleus accumbens (NAc) in the anorectic effects of 3,4-N-methylene-dioxymethamphetamine

(MDMA, the active principle of ecstasy). Direct activation of 5-HT₄ receptors of the NAc reduced the physiological drive to eat in food deprived mice and increases the synthesis of the CART peptide (CART:cocaine- and amphetamine-regulated transcript) via a cAMP/PKA signalling pathway

- *11. Compan V, Zhou M, Grailhe R, Gazzara RA, Martin R, Gingrich J, Dumuis A, Brunner D, Bockaert J, Hen R: **Attenuated response to stress and novelty and hypersensitivity to seizures in 5-HT₄ receptor knock-out mice.** *J Neurosci* 2004, **24**:412-419.

Generation of 5-HT₄ receptor knock-out mice and description of the first phenotypes.

- *12. Bockaert J, Perroy J, Becamel C, Marin P, Fagni L: **GPCR interacting proteins (GIPs) in the nervous system: Roles in physiology and pathologies.** *Annu Rev Pharmacol Toxicol* 2010, **50**:89-109.

A review analysing the nature, the physiological functions, pathological implications and the putative roles as drug targets of GPCR interacting proteins (GIPs).

- **13. Alexander B, Warner-Schmidt J, Eriksson T, Tamminga C, Arango-Llievano M, Ghose S, Vernov M, Stavarche M, Musatov S, Flajolet M, et al.: **Reversal of depressed behaviors in mice by p11 gene therapy in the nucleus accumbens.** *Sci Transl Med* 2010, **2**:54ra76.

A provocative report describing, in mice, data supporting a brain region-specific gene therapy for depression: adeno-associated virus (AAV)-mediated transfer of the gene encoding p11 to the NAc. p11 interacts with several channels, 5-HT_{1B} and 5-HT₄ receptors increasing their cell surface expression. p11 is increased in rodent brain following antidepressant and electroconvulsive therapies. Here, the authors found that local NAc knockdown of p11 resulted in behavioural deficits related to depression and that AAV-mediated p11 gene transfer to the NAc rescued the depression-related behaviours in p11 knock-out mice. Is gene therapy for severe psychiatric disorders a reasonable hope?

- *14. Barthet G, Framery B, Gaven F, Pellissier L, Reiter E, Claeysen S, Bockaert J, Dumuis A: **5-hydroxytryptamine 4 receptor activation of the extracellular signal-regulated kinase pathway depends on Src activation but not on G protein or beta-arrestin signaling.** *Mol Biol Cell* 2007, **18**:1979-1991.

5-HT₄ receptors, as many GPCRs, can signal without activation of a G protein. Many GPCRs activate ERK pathway in a non-G but β -arrestin dependent manner whereas 5-HT₄ receptors activate this pathway via the activation of Src and without the implication of β -arrestin.

15. Woehler A, Ponimaskin EG: **G protein--mediated signaling: same receptor, multiple effectors.** *Curr Mol Pharmacol* 2009, **2**:237-248.

16. Gill RK, Saksena S, Tyagi S, Alrefai WA, Malakooti J, Sarwar Z, Turner JR, Ramaswamy K, Dudeja PK: **Serotonin inhibits Na⁺/H⁺ exchange activity via 5-HT₄ receptors and activation of PKC alpha in human intestinal epithelial cells.** *Gastroenterology* 2005, **128**:962-974.

- **17. Barthet G, Carrat G, Cassier E, Barker B, Gaven F, Pillot M, Framery B, Pellissier LP, Augier J, Kang DS, et al.: **Beta-arrestin1 phosphorylation by GRK5 regulates G protein-independent 5-HT₄ receptor signalling.** *Embo J* 2009, **28**:2706-2718.

The first report on the mechanisms of desensitization of a non-G, non- β -arrestin dependent signaling of a GPCR, the 5-HT₄ receptor-mediated activation of the Src/ERK pathway.

18. Joubert L, Hanson B, Barthet G, Sebben M, Claeysen S, Hong W, Marin P, Dumuis A, Bockaert J: **New sorting nexin (SNX27) and NHERF specifically interact with the 5-HT_{4a} receptor splice variant: roles in receptor targeting.** *J Cell Sci* 2004, **117**:5367-5379.

19. Svenningsson P, Chergui K, Rachleff I, Flajolet M, Zhang X, El Yacoubi M, Vaugeois JM, Nomikos GG, Greengard P: **Alterations in 5-HT_{1B} receptor function by p11 in depression-like states. *Science* 2006, **311**:77-80.

p11, a member of the S100 EF-hand protein family is shown to interact with 5-HT_{1B} receptors increasing their cell surface expression. p11 is increased in rodent brain following antidepressant and electroconvulsive therapies but decreased in an animal model of depression and in brain tissues of depressed patients. Over-expression of p11 recapitulated certain behaviours of antidepressant in mice whereas p11 knock-out mice exhibit a depression-like phenotype.

*20. Warner-Schmidt JL, Flajolet M, Maller A, Chen EY, Qi H, Svenningsson P, Greengard P: **Role of p11 in cellular and behavioral effects of 5-HT₄ receptor stimulation.** *J Neurosci* 2009, **29**:1937-1946.

p11 is a GIP of 5-HT₄ receptors enhancing their membrane trafficking and signalling.

21. Becamel C, Alonso G, Galeotti N, Demey E, Jouin P, Ullmer C, Dumuis A, Bockaert J, Marin P: **Synaptic multiprotein complexes associated with 5-HT(2C) receptors: a proteomic approach.** *Embo J* 2002, **21**:2332-2342.

22. Becamel C, Gavarini S, Chanrion B, Alonso G, Galeotti N, Dumuis A, Bockaert J, Marin P: **The serotonin 5-HT_{2A} and 5-HT_{2C} receptors interact with specific sets of PDZ proteins.** *J Biol Chem* 2004, **279**:20257-20266.

*23. Lezoualc'h F, Robert SJ: **The serotonin 5-HT₄ receptor and the amyloid precursor protein processing.** *Exp Gerontol* 2003, **38**:159-166.

Review in favor of the role of 5-HT₄ receptors in stimulating the non-amyloid APP (amyloid precursor protein) metabolism.

*24. Huang YY, Kandel ER: **5-Hydroxytryptamine induces a protein kinase A/mitogen-activated protein kinase-mediated and macromolecular synthesis-dependent late phase of long-term potentiation in the amygdala.** *J Neurosci* 2007, **27**:3111-3119.

5-HT₄ receptors are stimulating long term potentiation (LTP) in amygdala.

25. Kemp A, Manahan-Vaughan D: **Hippocampal long-term depression and long-term potentiation encode different aspects of novelty acquisition.** *Proc Natl Acad Sci U S A* 2004, **101**:8192-8197.

26. Mlinar B, Mascalchi S, Mannaioni G, Morini R, Corradetti R: **5-HT₄ receptor activation induces long-lasting EPSP-spike potentiation in CA1 pyramidal neurons.** *Eur J Neurosci* 2006, **24**:719-731.

27. Levallet G, Hotte M, Boulouard M, Dauphin F: **Increased particulate phosphodiesterase 4 in the prefrontal cortex supports 5-HT₄ receptor-induced improvement of object recognition memory in the rat.** *Psychopharmacology (Berl)* 2009, **202**:125-139.
- **28. Restivo L, Roman F, Dumuis A, Bockaert J, Marchetti E, Ammassari-Teule M: **The promnesic effect of G-protein-coupled 5-HT₄ receptors activation is mediated by a potentiation of learning-induced spine growth in the mouse hippocampus.** *Neuropsychopharmacology* 2008, **33**:2427-2434.
The 5-HT₄ receptor partial agonist SL65.0155 enhanced simultaneous olfactory discrimination performance and potentiates learning-induced dendritic spine growth in the mouse hippocampus.
29. Segu L, Lecomte MJ, Wolff M, Santamaria J, Hen R, Dumuis A, Berrard S, Bockaert J, Buhot MC, Compan V: **Hyperfunction of muscarinic receptor maintains long-term memory in 5-HT₄ receptor knock-out mice.** *PLoS One* 2010, **5**:e9529.
30. Cachard-Chastel M, Lezoualc'h F, Dewachter I, Delomenie C, Croes S, Devijver H, Langlois M, Van Leuven F, Sicsic S, Gardier AM: **5-HT₄ receptor agonists increase sAPP α levels in the cortex and hippocampus of male C57BL/6j mice.** *Br J Pharmacol* 2007, **150**:883-892.
31. Mohler EG, Shacham S, Noiman S, Lezoualc'h F, Robert S, Gastineau M, Rutkowski J, Marantz Y, Dumuis A, Bockaert J, et al.: **VRX-03011, a novel 5-HT₄ agonist, enhances memory and hippocampal acetylcholine efflux.** *Neuropharmacology* 2007, **53**:563-573.
32. Reynolds GP, Mason SL, Meldrum A, De Keczer S, Parnes H, Eglen RM, Wong EH: **5-Hydroxytryptamine (5-HT)₄ receptors in post mortem human brain tissue: distribution, pharmacology and effects of neurodegenerative diseases.** *Br J Pharmacol* 1995, **114**:993-998.
33. Wong EH, Reynolds GP, Bonhaus DW, Hsu S, Eglen RM: **Characterization of [3H]GR 113808 binding to 5-HT₄ receptors in brain tissues from patients with neurodegenerative disorders.** *Behav Brain Res* 1996, **73**:249-252.
34. Tsang SW, Keene J, Hope T, Spence I, Francis PT, Wong PT, Chen CP, Lai MK: **A serotonergic basis for hyperphagic eating changes in Alzheimer's disease.** *J Neurol Sci* 2010, **288**:151-155.
35. Rybkin, II, Zhou Y, Volaufova J, Smagin GN, Ryan DH, Harris RB: **Effect of restraint stress on food intake and body weight is determined by time of day.** *Am J Physiol* 1997, **273**:R1612-1622.
36. Wagner A, Aizenstein H, Venkatraman VK, Bischoff-Grethe A, Fudge J, May JC, Frank GK, Bailer UF, Fischer L, Putnam K, et al.: **Altered striatal response to reward in bulimia nervosa after recovery.** *Int J Eat Disord* 2010, **43**:289-294.
37. Asakawa A, Ueno N, Katagi M, Ijuin Y, Morita Y, Mizuno S, Inui T, Sakamaki R, Shinfuku N, Uemoto M: **Mosapride improves food intake, while not worsening**

glycemic control and obesity, in ob/ob obese mice with decreased gastric emptying. *J Diabetes Complications* 2006, **20**:56-58.

38. Kornum BR, Lind NM, Gillings N, Marnier L, Andersen F, Knudsen GM: **Evaluation of the novel 5-HT₄ receptor PET ligand [11C]SB207145 in the Gottingen minipig.** *J Cereb Blood Flow Metab* 2009, **29**:186-196.

39. Ge J, Barnes NM: **5-HT₄ receptor-mediated modulation of 5-HT release in the rat hippocampus in vivo.** *Br J Pharmacol* 1996, **117**:1475-1480.

40. Lucas G, Debonnel G: **5-HT₄ receptors exert a frequency-related facilitatory control on dorsal raphe nucleus 5-HT neuronal activity.** *Eur J Neurosci* 2002, **16**:817-822.

41. Conductier G, Dusticier N, Lucas G, Cote F, Debonnel G, Daszuta A, Dumuis A, Nieoullon A, Hen R, Bockaert J, et al.: **Adaptive changes in serotonin neurons of the raphe nuclei in 5-HT(4) receptor knock-out mouse.** *Eur J Neurosci* 2006, **24**:1053-1062.

*42. Lucas G, Compan V, Charnay Y, Neve RL, Nestler EJ, Bockaert J, Barrot M, Debonnel G: **Frontocortical 5-HT₄ receptors exert positive feedback on serotonergic activity: viral transfections, subacute and chronic treatments with 5-HT₄ agonists.** *Biol Psychiatry* 2005, **57**:918-925.

Intraperitoneal injections but not local microiontophoretic application of 5-HT₄ receptor agonists on dorsal raphe (DR) 5-HT neurons augmented the firing of a subpopulation of neurons. Thus, the implicated 5-HT₄ receptors are likely localized outside of DR. The authors showed that microinjections of Herpes simplex viruses, transformed to express 5-HT₄ receptors, increased DR 5-HT neuronal activity when performed in the medial prefrontal cortex (mPFC) but not in others brain areas suggesting a fronto-cortical positive feedback on DR 5-HT neurons.

*43. Cai X, Flores-Hernandez J, Feng J, Yan Z: **Activity-dependent bidirectional regulation of GABA(A) receptor channels by the 5-HT(4) receptor-mediated signalling in rat prefrontal cortical pyramidal neurons.** *J Physiol* 2002, **540**:743-759.

An electrophysiology and cellular biology paper establishing the expression of 5-HT₄ receptors on pyramidal cortical neurons and the regulation of GABA_A receptor channels.

44. Kobayashi K, Ikeda Y, Sakai A, Yamasaki N, Haneda E, Miyakawa T, Suzuki H: **Reversal of hippocampal neuronal maturation by serotonergic antidepressants. *Proc Natl Acad Sci U S A* 2010, **107**:8434-8439.

In hippocampus and granular cells (GCs) 5-HT₄ receptors potentiated mossy-fiber-CA3 synaptic transmission. This potentiation is increased or decreased after chronic treatments with low and high doses of fluoxetine, respectively. The increase in synaptic potentiation is proposed to be the result of a GCs "dematuration" induced by fluoxetine via activation of 5-HT₄ receptors.

45. Licht CL, Marcussen AB, Wegener G, Overstreet DH, Aznar S, Knudsen GM: **The brain 5-HT₄ receptor binding is down-regulated in the Flinders Sensitive Line depression model and in response to paroxetine administration.** *J Neurochem* 2009, **109**:1363-1374.

46. Vidal R, Valdizan EM, Mostany R, Pazos A, Castro E: **Long-term treatment with fluoxetine induces desensitization of 5-HT4 receptor-dependent signalling and functionality in rat brain.** *J Neurochem* 2009, **110**:1120-1127.
47. Compan V, Daszuta A, Salin P, Sebben M, Bockaert J, Dumuis A: **Lesion study of the distribution of serotonin 5-HT4 receptors in rat basal ganglia and hippocampus.** *Eur J Neurosci* 1996, **8**:2591-2598.
48. Roychowdhury S, Haas H, Anderson EG: **5-HT1A and 5-HT4 receptor colocalization on hippocampal pyramidal cells.** *Neuropharmacology* 1994, **33**:551-557.
49. Vilaro MT, Cortes R, Mengod G: **Serotonin 5-HT4 receptors and their mRNAs in rat and guinea pig brain: distribution and effects of neurotoxic lesions.** *J Comp Neurol* 2005, **484**:418-439.
50. Waeber C, Sebben M, Nieoullon A, Bockaert J, Dumuis A: **Regional distribution and ontogeny of 5-HT4 binding sites in rodent brain.** *Neuropharmacology* 1994, **33**:527-541.

Figure legends

Figure 1: G_s-independent signalling and desensitization of 5-HT₄ receptors.

In addition to G proteins (mainly G_s) activation, 5-HT₄ receptors activated the tyrosine kinase Src in a G-protein independent manner [13*]. Src is associated with the inactive 5-HT₄ receptors and phosphorylated, therefore activated, upon receptor agonist stimulation. Activation of Src led to a β-arrestin-independent ERK activation in neurons or PLCγ₁ activation and finally downstream Na⁺/H⁺ exchanger inhibition in intestinal cells [16]. Desensitization of the 5-HT₄ receptor-induced Src activation first involved GRK5 recruitment. GRK5 phosphorylated β-arrestin. Phosphorylated β-arrestin inhibited, via an unknown mechanism the 5-HT₄ receptor-induced Src phosphorylation [17**].

Figure 2: Cellular localization of 5-HT₄ receptors in the forebrain.

5-HT₄ receptors are mainly located on neurons containing γ-aminobutyric acid (GABA) in the nucleus accumbens and striatum [47], pyramidal cells expressing the glutamate (GLU) in the medial prefrontal cortex [43*] and hippocampus (CA1 [48], CA3: [49] and granule cells [44**,49]).

5-HT₄ receptors are unlikely located on dorsal raphe nucleus (DRN) [49] (Compan, unpublished). However, autoradiographic studies have shown moderated densities of 5-HT₄ receptors in the rat DRN, using both the radiolabeled antagonists [³H]GR 113808 [50] or [¹²⁵I]SB207710 [49] suggesting that these receptors are possibly localized on DRN afferent neurons. 5-HT₄ receptors are heteroreceptors, located on the somatodendritic and axon terminals of the spiny efferent neurons of the striatum containing GABA to the substantia nigra and globus pallidus [47]. 5-HT₄ receptors are not located on the nigro-striatal dopaminergic neurons [47].

In brain areas of the reward system, 5-HT₄ receptors are located on the pyramidal cells of the medial prefrontal cortex [43*] that also expressed 5-HT_{1A} and 5-HT_{2A} receptors. More recently, it has been shown that 5-HT₄ receptors are likely expressed by efferent neurons of the nucleus accumbens to the lateral hypothalamus [10**].

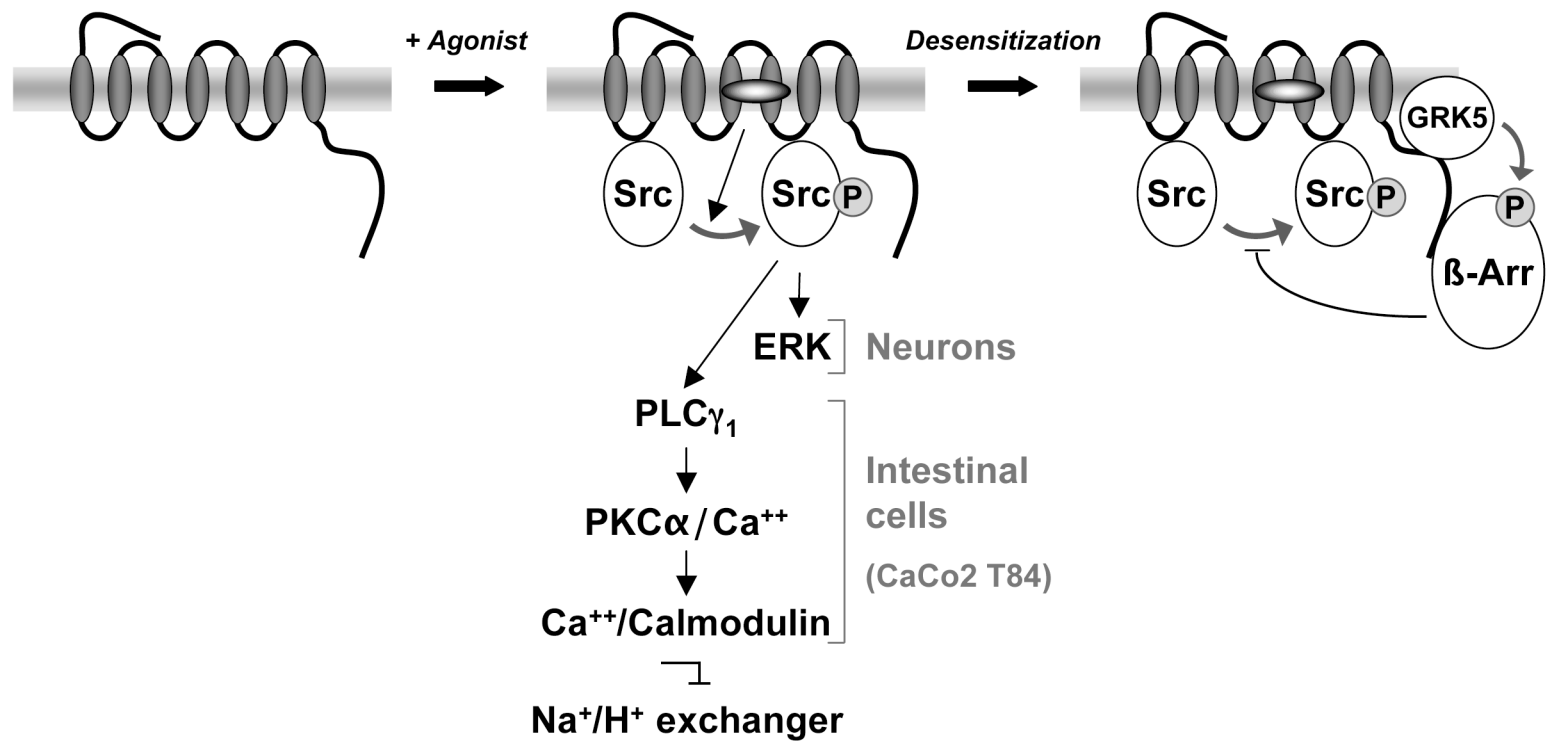


Figure 1

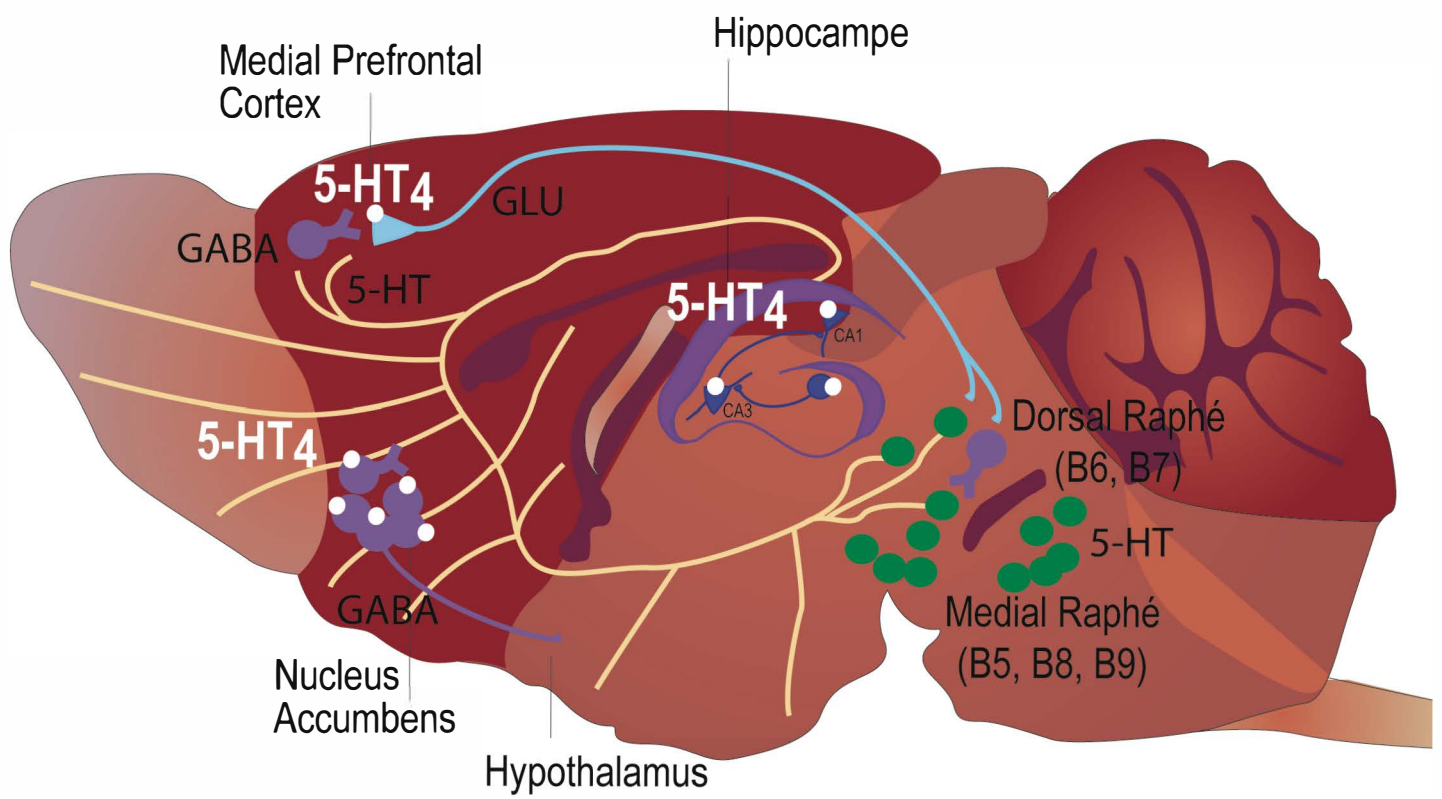


Figure 2