Review

Alterations of Expression of the Serotonin 5-HT4 Receptor in Brain Disorders

Heike Rebholz 1,*, Eitan Friedman 1,2 and Julia Castello 1,2

1 Department of Molecular, Cellular and Biomedical Sciences, CUNY School of Medicine, New York, NY 10031, USA; Friedman@med.cuny.edu (E.F.); julia.csaval@gmail.com (J.C.)
2 Ph.D. Programs in Biochemistry and Biology, The Graduate Center, City University of New York, New York, NY 10031, USA
* Correspondence: heikerebholz@gmail.com; Tel.: +1-212-650-8283

Received: 14 October 2018; Accepted: 6 November 2018; Published: 13 November 2018

Abstract: The serotonin 4 receptor, 5-HT4R, represents one of seven different serotonin receptor families and is implicated in a variety of physiological functions and their pathophysiological variants, such as mood and depression or anxiety, food intake and obesity or anorexia, or memory and memory loss in Alzheimer’s disease. Its central nervous system expression pattern in the forebrain, in particular in caudate putamen, the hippocampus and to lesser extent in the cortex, predispose it for a role in executive function and reward-related actions. In rodents, regional overexpression or knockdown in the prefrontal cortex or the nucleus accumbens of 5-HT4R was shown to impact mood and depression-like phenotypes, food intake and hypophagia; however, whether expression changes are causally involved in the etiology of such disorders is not clear. In this context, more data are emerging, especially based on PET technology and the use of ligand tracers that demonstrate altered 5-HT4R expression in brain disorders in humans, confirming data stemming from post-mortem tissue and preclinical animal models. In this review, we would like to present the current knowledge of 5-HT4R expression in brain regions relevant to mood/depression, reward and executive function with a focus on 5-HT4R expression changes in brain disorders or caused by drug treatment, at both the transcript and protein levels.

Keywords: serotonin; 5-HT 4 receptor; 5-HT4R; depression; mood disorder; expression; Alzheimer’s disease; cognition; Parkinson’s disease

1. Introduction

5-HT receptors are composed of 7 families (5-HT1-7 receptors), comprising 14 structurally and pharmacologically distinct 5-HT receptor subtypes [1]. All receptors are G-protein-coupled, with the exception of the 5-HT3R that belongs to the superfamily of ligand-gated ion channels. Members of all 7 receptor families are expressed in the brain: 5-HT1 receptors are \(G_{\alpha_0/11}\)-coupled and two receptors of this family, 5-HT1aR and 5-HT1bR, have an important function as somatodendritic autoreceptors expressed on neurons of the raphe nuclei that produce 5-HT, but they are also expressed as postsynaptic heteroreceptors in several brain areas [2]. The three members of the \(G_{\alpha_5/11}\)-coupled 5-HT2R family have well defined roles in the periphery such as in the vascular system and muscle contraction; however, their function in the brain is not well understood A potential link between a 5-HT2C receptor and vulnerability to affective disorders has been reported, and a number of antipsychotics have inverse agonist activity at 5-HT2C receptors [3]. 5-HT2C KO mice are highly obese and suffer from epilepsies [4]. 5-HT2aR mediates the hallucinatory and psychotic action of psychedelic drugs such as LSD or psilocybin [5]. Human brain \(G_{\alpha_5/11}\)-coupled 5-HT3R expression is localized to the cerebral cortex, hippocampus, cerebellum, and a role in mood and major depression was
postulated, using pharmacological tools and knockout mice [6]. 5-HT_{6}Rs are postsynaptic G_{\alpha}s-coupled receptors strongly expressed in the striatum, nucleus accumbens and cortex, and moderately in the hippocampus, amygdala, and hypothalamus. They control among others central cholinergic function [6]. The G_{\alpha}s-coupled 5HT_{7}R is mainly expressed in the limbic system, and a potential role in sleep, circadian rhythmic activity and mood has been suggested [6]. Among all 5-HT receptors, the type 3 receptor is the only ligand-gated ion channel receptor triggering rapid depolarization via the opening of non-selective cation channels. 5-HT_{3}R expression in the forebrain is low, but higher levels are present in the hippocampus and amygdala [6].

5-HT_{4}R was initially identified in cultured mouse colliculi cells and guinea pig brain using a functional cAMP stimulation assay [7]. In 1995, its cloning was reported [8]. Two different splicing variants, a short one, found in the striatum and a long one in the whole brain [8] were initially described, while others found the short form also present more universally in the brain [9].

Expression in the brain is greatest in the basal ganglia, the hippocampal formation and the cortex, as shown in human and rat brain [10,11]. 5-HT_{4}R is also widely distributed in the body. Outside the CNS, it is found along the gastrointestinal tract (esophagus, ileum and colon) [12]. It is also present in the bladder, the heart and the adrenal glands. 5-HT_{4} receptors are well known for their peripheral effects on the gastrointestinal tract, and are targets in the treatment of dyspepsia, gastroesophageal reflux disease, gastroparesis or irritable bowel syndrome [13,14]. Serotonin affects heart contractility through 5-HT_{4}R which is expressed in the human and pig atrium and ventricle, while, interestingly, in the rat it is only expressed in the atrium [15]. 5-HT_{4}R activation leads to the contraction of heart but also to tachycardia and arrhythmia [15]. The cardiac contractile effects of 5-HT_{4}R are restricted to human and pig atria and are absent from a large number of laboratory animals, such as rat, guinea pig, rabbit and frog [16]. 5-HT_{4}R was also shown to be overexpressed in the cortex of the adrenal gland of a subtype of Cushing syndrome patients, a condition caused by cortisol hyper-production [17].

Compared to other serotonin receptors, the gene encoding 5-HT_{4}R (htr4) is large and its architecture is complicated, with 38 exons spaced over 700 kb [18]. As a G-protein coupled receptor, 5-HT_{4}R signals through both G protein-dependent and G protein-independent pathways. The major G protein engaged by 5-HT_{4}R signaling is G_{\alpha}s, leading to the activation of the cAMP/PKA pathway [19]. The G-protein independent non-canonical pathway activates Src and ERK kinases, leading to pERK1/2 phosphorylation [20].

5-HT_{4}R KO mice develop normally, with no differences in body weight, metabolism, social behavior, or sleep pattern [21]. However, when stressed they exhibit reduced hypophagia [21] and re-expression of 5-HT_{4}R in the medial prefrontal cortex rescues this phenotype [22]. In mice, 5-HT_{4}R was also shown to link appetite and feeding to addiction-related behaviors since 5-HT_{4}R activation in the nucleus accumbens provokes anorexia and hyperactivity, concurrently upregulating a gene induced by cocaine and amphetamine (CART) while knockdown thereof inhibits MDMA-induced hyperactivity [23].

One of the earliest functions attributed to 5-HT_{4}R in rodents is its excitatory effect on acetylcholine release in the frontal cortex and the hippocampus [24,25] which was linked to its role in enhancing memory and cognition [26–29]. For example, a two-week treatment with 5-HT_{4}R partial agonist RS67333 improved memory in the object recognition test in mice [30]. Olfactory associative learning was enhanced by another partial agonist (SL65.0155) in rats [31]. Other paradigms assessing social memory, autoshaping and spatial and place learning, showed a memory enhancing effect of 5-HT_{4}R stimulation [29,32,33]. Conversely, receptor antagonists induced a consistent deficit in (olfactory) associative memory formation [34,35], and weakened passive avoidance memory [36]. Paralleling these behavioral changes are structural plasticity effects of potentiated learning-induced dendritic spine growth in the hippocampus in mice, an effect which is abolished by 5-HT_{4}R inhibition [37].

5-HT_{4} receptors have also been found to modulate GABA and dopamine release [18,26]. Serotonin depolarizes globus pallidus neurons, increases their firing rate and alters GABA release in a 5-HT_{4}R-dependent manner involving pre- and postsynaptic mechanisms [38]. In guinea pig,
the 5-HT_4R agonist BIMU-8 increased GABA release from hippocampus indirectly via cholinergic muscarinic receptors [39]. 5-HT_4 receptors exert excitatory control on DA release in the striatum, while a receptor antagonist blocks this effect [40]. In freely moving rats, the 5-HT_4R antagonist GR125487 significantly reduced the nigrostriatal haloperidol-induced but not basal DA outflow without affecting the mesoaccumbal DA release, indicating that 5-HT_4R exerts facilitatory control under activated conditions [41]. This finding is also important in the context of Parkinson’s disease where the substantia nigra is selectively vulnerable to degeneration compared to the VTA, leading to a depletion in striatal dopamine. In a rat model of PD, the 5-HT_4R agonist prucalopride selectively enhanced L-DOPA-stimulated DA release in the rat SNr and the PFC but not in the hippocampus or the striatum [42].

5-HT_4R also impacts global serotonergic tone. 5-HT_4R KO mice have diminished tissue levels of 5-HT and its main metabolite, 5-HIAA, increased serotonin transporter (SERT) at the protein and transcript levels, as well as decreased 5-HT_1A binding sites [43]. 5-HT_4R is a component of a feedback loop projecting from the PFC to the dorsal raphe nuclei (DRN). More specifically, in mice, systemic 5-HT_4R stimulation or overexpression of 5-HT_4R in the mPFC increased the firing rate of DRN neurons, thus creating a positive feedback PFC-DRN loop involving 5-HT_4R activation in cortical projections neurons, glutamate release in the DRN and enhanced DRN firing [44–46].

5-HT_4R is a major candidate in mediating antidepressant drug action. As early as 1997, a role of 5-HT_4R in anxiety-like behavior was described in rats [47]. More recently, this topic has received more interest, possibly due to the need to identify novel, fast acting antidepressant drugs. Indeed, it was described in rodents that subchronic (3 days) treatment with 5-HT_4R agonist yields behavioral as well as biochemical responses in the hippocampus (CREB phosphorylation, neurogenesis) that are comparable to responses to treatment with SSRIs over 3 weeks [48], possibly through its action in the above mentioned PFC-DRN feedback loop [44–46].

These findings clearly indicate that 5-HT_4R is a major regulator of the homeostasis of several neurotransmitter systems, implying a role in brain disorders such as Alzheimer’s, Huntington’s, Parkinson’s diseases or Major Depressive Disorder. Our review aims at summarizing the current knowledge of 5-HT_4R expression in the brain. We also want to present knowledge on cell-type specific expression, which has not yet been studied extensively, partly due to the lack of immunohistochemistry-competent antibodies as well as resolution limits of binding experiments in brain slices with radioactive antagonists.

2. Promoter Studies and Transcript Variants

Surprisingly little is known about the transcriptional regulation of the htr4 gene across tissues. The human 5-HT_4 receptor gene is located on chromosome 5 (5q31–q33) and contains five exons and eight alternatively spliced cassettes that code for the internal and C-terminal splice variants [16]. Human htr4 mRNA is transcribed from a very complex gene encompassing 38 exons spanning over 700 kb [18], and multiple C-terminal isoforms are expressed in specific tissues in the CNS. To date, it is not known how 5-HT_4 receptor expression is regulated in the brain, and so far we have only partial knowledge about the promoter, derived from human atrial tissue and placenta [16,49]. In the heart, the major transcription start site of the htr4 gene is located at −3185 bp upstream of the first start codon [16]. In placenta, the 5′-UTR is even longer, spanning over 5100 bp upstream from the translation start site [49]. The different 5′-UTRs upstream of the translation initiation codon are interesting since they may hold an additional key to understand region and cell-type specific regulation of protein expression.

The human promoter lacks TATA and CAAT canonical motifs, but contains several transcription factors binding sites. Transient transfection assays with human 5-HT_4 receptor promoter-luciferase constructs identified an approx. 1.2 kb fragment of 5′-non-transcribed sequence as promoter in human cell lines but not monkey COS-7 cells [16] indicating that there is a tissue-specific expression of yet unknown transcription factors. We found in mouse brain that there is a region-specific negative transcriptional regulation of htr4 exerted by the kinase CK2. Examination of conditional mouse
knockouts of CK2 in the hippocampus, striatum and the cortex indicated an upregulation of 5-HT$_4$R mRNA selectively in the cortex [46]. Furthermore, in luciferase assays, using a 4 kb element upstream of the mouse gene fused to luciferase cDNA, expression was promoted when CK2 was inhibited or knocked down in human HeLa cells but not in Hek293 or monkey COS-7 cells, again underlining the importance of tissue-specific transcription factors.

Instead of the TATA box, Maillet et al. described the presence of a sequence in the human gene (TTCACCTTT) that can function as a core promoter sequence similarly to the TATA box [16]. For other species, no promoter studies were performed.

There are differential transcription initiation sites in different tissues such as human heart and placenta while for the brain no such data are yet available. While the transcription initiation start site does not affect the protein-coding region, it may alter the transcription efficiency and the expression pattern of 5-HT$_4$R. It is hypothesized that such a long 5'-UTR reduces RNA translation and leads to low levels of expressed transcripts by causing premature initiation at a wrong ATG and preventing the ribosome from reaching the correct start codon [16].

Taken together, in particular in the human brain, there is a lack of data about the 5'-UTR, the promoter and the transcription factors that are active at the promoter for htr4.

3. SNPs in Non-Coding Regions

In addition to the 5'-UTRs, isoforms can also vary in the 3'-UTR. These 3'-UTRs are targets for post-transcriptional regulation by non-coding RNAs such as miRNAs. Within the 3'-UTR of the 5-HT$_4$R (b) and (i) isoforms from the GI tract from humans with irritable bowel syndrome (IBS), a single nucleotide polymorphism, termed 5-HT$_4$R (b_2) was found to be predominantly present in a subtype of IBS patients. This isoform lacks two of the three miRNA binding sites for miR-16 family/miR-103/107 and, compared to the full length 5-HT$_4$R (b) isoform, its expression yielded higher 5-HT$_4$R protein levels. It was further shown that miR-16 and miR-103 are responsible for the downregulation of the transcript in vitro which is impaired in the 5-HT$_4$R (b_2) mutant [50].

Another miRNA, Let-7a, was also postulated to have the potential to regulate 5-HT$_4$R [51].

Several genome wide association studies (GWAS) and meta-analyses have associated twelve intronic SNPs in the non-coding region of human htr4 with pulmonary function [52,53]. The same SNPs have been associated with the clinical phenotypes of airflow obstruction and COPD and asthma [53,54]. A SNP in a non-coding region could affect transcriptional regulation or generate a splicing signal. In this context, the pulmonary function of 5-HT$_4$R KO mice was found to exhibit higher baseline lung resistance, confirming a role of 5-HT$_4$R in airway diseases [55]. No mechanistic studies have yet been performed to understand the impact of the described SNP on transcription and splicing.

4. Isoforms and Alternative Splicing

In contrast to promoter-dependent transcriptional initiation sites which will still yield the same transcript but alter expression levels, splicing affects the protein sequence.

Since the first publication in 1995 which described a short and a long isoform, several other isoforms were discovered: There are at least 11 human 5-HT$_4$ receptor splice variants (a–i,n) [18,56–59]. All splice variants differ at the C-terminus with the exception of 5-HT$_4$R (h) which is an internal splice variant with an insert in the 2nd extracellular loop [60], (Figure 1) and the (n) isoform which lacks the C-terminal exon [61].

Human 5-HT$_4$ receptor isoforms (a–i and n) are highly expressed in the central nervous system [18,56,61]. Isoform (b) is the most abundant form in the CNS and periphery, and is expressed in the caudate nucleus, putamen, amygdala, pituitary gland, and small intestine. Isoform (a) is highly expressed in the amygdala, hippocampus, nucleus accumbens, and caudate nucleus and at lower levels in the small intestine, the atrium, and pituitary gland. Isoform (c) is highly expressed in the pituitary gland and small intestine and to a lesser degree in the caudate nucleus, hippocampus, and putamen. Isoform (d) is not present in the CNS but is found in the small intestine [18,61,62].
Isoform (g) seems to be highly expressed in the hypothalamus and cortex [63]. The (n) variant, which lacks the alternatively spliced C-terminal exon, is abundantly expressed in human peripheral tissues and brain regions involved in mood disorders (frontal cortex, hippocampus) [61].

Mice are currently thought to have five [64] and rats four isoforms, with the fourth, (c1) isoform expressed in the gastrointestinal tract [59, 63]. In rat brain, no significant difference in expression between the long and short variants has been found by ISH [65]. The C-terminal sequences will determine the baseline activity (with the shorter isoforms being more active) or the ability to recruit binding partners such as β-arrestins and GRKs, sorting nexins or the NHERF PDZ adaptor protein [19, 66, 67]. This will affect internalization kinetics which are different between isoforms [68]. Finally, isoforms can differ in their G protein coupling, since the 5-HT4R (b) isoform can couple via Gαq as well as Gαs [69].

To date, no specific isoform has been linked to a brain disorder; however, an interaction cannot be excluded since such studies have not been performed and would be very challenging. Most human studies using PET technology or radioactive labeling are based on ligands which cannot distinguish between isoforms. Quantitative RT-PCR was used to detect different isoforms and their expression in the rodent brain; however, no studies in disease models have employed this approach. The fact that mice or rats do not express the same isoforms than humans suggests that fine tuning of 5-HT4 signaling through a differential expression of longer or short, more active versus less active isoforms, may occur in different species.

Figure 1. Alignment of C-termini of isoforms found in human tissue: green: leucine 358, the last amino acid common to all variants. For the c isoform, a short and a long one were described. Yellow: S/T cluster necessary for β-arrestin dependent receptor endocytosis.

5. Post-Translational Regulation

5.1. Phosphorylation

The amount of membrane-localized and active GPCR is a result of the ratio between receptor endocytosis and recycling. Endocytosis is initiated through (S/T) phosphorylation of GPCRs in their intracellular domains by G protein-coupled receptor kinases (GRKs) and second messenger kinases such as PKA or PKC [70]. Binding of arrestins to GRK-phosphorylated receptors results in receptor desensitization [71] and internalization [72–76].

Fourteen phosphosites in the 3rd intracellular loop and in the C-terminal tail of 5-HT4R that was heterologously expressed in retinal rod cells of the mouse were identified [77]; however, the identity of the kinases has not been determined. Neither has it been tested whether the phosphorylation of these sites is activity dependent.

In Hek293 cells, it was shown that GRK2 phosphorylates and desensitizes 5-HT4R resulting in downregulation of the cAMP/PKA pathway, while GRK5-mediated 5-HT4R phosphorylation resulted in reduced inhibition of ERK phosphorylation [19, 78].

5.2. Palmitoylation

Palmitoylation is a lipid modification in which a cysteine SH group undergoes esterification with a palmitoyl group, generating an anchor to the lipid bilayer of the plasma membrane. This modification is readily reversible and, similar to phosphorylation/dephosphorylation, allows for rapid regulation of protein function, affecting GCPR endocytosis, phosphorylation, desensitization and ultimately
cellular signaling. Biochemical studies in insect (Sf9) and mammalian cells (Cos7) showed that several 5-HT receptors (5-HT₁₄R, 5-HT₁₅R, 5-HT₄R and 5-HT₇R) are palmitoylated in their C-terminal tails. The mouse 5-HT₄R (a) variant is palmitoylated at 3 highly conserved cysteine sites and at a C-terminal cysteine that is variant-specific. Palmitoylation near or close to protein-protein interaction motifs will affect the binding properties of the receptor, impact on constitutive activity or internalization via β-arrestin-2 [79,80].

5.3. Glycosylation

Only one study describes two putative N–linked glycosylation sites that conform to the consensus sequence N–X–S/T (X being any amino acid but proline) for glycosylation. These are located on the extracellular side of 5-HT₄R, one at the N-terminus and one in the 2nd extracellular loop [77].

What is clearly missing in our understanding of all described post-translational modifications are data generated from physiologically expressed 5-HT₄R such as in mouse brain, a comparison between brain regions and an analysis in response to drug treatment or of brain disease models. Finally, the functionality of each of these modifications should be addressed, in particular on their effect on protein stability and receptor homeostasis.

6. Basal Expression

6.1. Transcript Level

5-HT₄R transcript expression in rodents mainly stems from in situ hybridization (ISH) experiments: In rat brain slices, ISH probes showed strong expression in the basal ganglia (caudate putamen, ventral striatum), olfactory tubercle, medial habenula and hippocampal formation while none was detected in globus pallidus and substantia nigra [65]. Similarly, human postmortem brains showed highest levels of 5-HT₄ receptor mRNA in caudate nucleus, putamen, nucleus accumbens, and the hippocampal formation but none in globus pallidus and substantia nigra [10].

A brain-wide comprehensive appraisal of cell-specific expression is still warranted; however, some evidence has been published: Dual-label in situ hybridization for 5-HT₄R and neuronal markers suggests expression in basal forebrain GABAergic parvalbumin synthesizing and glutamatergic cells and in glutamatergic pyramidal neurons in the medial prefrontal cortex and hippocampus of rat and guinea pig (CA1, CA3) [62,81]. 5-HT₄R mRNA is present in 60% of rat PFC pyramidal neurons of the frontal cortex as assessed by single cell mRNA/cDNA profiling [82,83].

In rat hippocampal slices, the 5-HT₄R agonist, cisapride, leads to increased hippocampal pyramidal cell activity and serotonin release, indirectly indicating that 5-HT₄R is expressed in these cells [84].

6.2. Protein Level

Our knowledge on 5-HT₄R protein expression stems to a large degree from radioactive ligand binding studies which for the most part has mirrored results of ISH studies. Indeed, a large number of radioligands exist that are specific to 5-HT₄R.

High densities of [3H]-GR 113808 or [125I]-SB 207710 binding sites are present in the ventral and dorsal striatum, substantia nigra, globus pallidus and ventral pallidum, interpeduncular nucleus, islands of Calleja, and olfactory tubercle in guinea pig, mouse and rat brain, lower densities are found in the hippocampus, septal region, neocortex, amygdala and colliculi as well as habenular and several thalamic and hypothalamic nuclei [65,85–87]. [125I]-SB 207710 binding in the caudate putamen shows a rostrocaudal and mediolateral increasing gradient of receptor densities, paralleling that observed for mRNA localization [65].

Kainic acid injection into the caudate-putamen of rats to destroy GABAergic striatal projection neurons resulted in a dramatic decrease of radioactive ligand binding, suggesting that 5-HT₄R is expressed in these neurons [11]. Similarly, 6-OHDA-lesion of dopaminergic neurons did not lead to a reduction in radioactive ligand binding but only to increased binding in the caudate putamen and
globus pallidus. This allows the conclusion that 5-HT$_4$R expression does not occur in DA neurons of the SN [11]. These studies were confirmed by comparing ISH and radioligand labeling data: The presence of mRNA in the rat caudate putamen and its absence in substantia nigra pars compacta and the globus pallidus suggests again that receptors found in binding studies in the caudate putamen and globus pallidus are synthesized by striatonigral and striatopallidal cells [62]. Comparison of mRNA distribution with receptor distribution as visualized with [125I]-SB 207710 further indicates that 5-HT$_4$ receptors are localized somatodendritically (e.g., in caudate putamen) and on axon terminals (e.g., in substantia nigra and globus pallidus) [65,88].

Transgenic Bac-GFP mice where GFP is expressed under the 5-HT$_4$R promoter are enabling a highly detailed look at protein expression in individual cells and confirm moderate to strong expression in the olfactory bulb, cerebral cortex, subicular cortex, hippocampus, striatum, globus pallidus, midbrain, pons medulla, cerebellum and weak expression in the piriform cortex, basal forebrain and the thalamus (Gensat Founder AU103). Dual immunohistochemical analysis showed expression of GFP in GABAergic spiny projection neurons but not in striatal interneurons [89]. Another transgenic mouse line where the β-galactosidase gene was knocked-in at the htr4 gene locus shows LacZ localization in mature but not immature granule cells as suggested by staining with the neural marker, NeuN, and calbindin (mature granule cell marker) [90]. Another study confirmed that 5-HT$_4$ receptors are expressed in efferent GABAergic neurons of the nucleus accumbens projecting to the lateral hypothalamus [23].

Species-specific differences of 5-HT$_4$R protein expression were found between mouse/rat and guinea pig in the globus pallidus, substantia nigra and interpeduncular nucleus [87].

Using [3H]-prucalopride and [3H]-GR116712 or [125I]-SB 207710 in binding studies of human post-mortem brain slices, the highest densities were found in the basal ganglia (caudate nucleus, putamen, nucleus accumbens, globus pallidus, substantia nigra). Moderate to low densities were detected in the hippocampal formation and in the cortical mantle [10]. Additionally, using the labeled antagonist GR 113808, expression in the human amygdala was reported [91]. In the neocortex, the binding showed a distinct lamination pattern with high levels in superficial layers and a band displaying lower levels in deep cortical layers [92]. Membrane binding studies with [3H]-GR 113808 resulted in highest binding in the human caudate nucleus, followed by substantial densities in the lenticular nucleus, the substantia nigra, the hippocampus and frontal cortex, whereas no binding could be detected in the cerebellum [82].

The expression data from all species studied are summarized in Table 1.

7. Changes in Expression in Brain Disorders and Changes Induced by Drug Treatment

In the healthy population there is a baseline difference of 5-HT$_4$R protein expression between sexes. Women show lower 5-HT$_4$R binding (by 13%) in the limbic system and the difference was most pronounced in the amygdala, which is highly involved in the processing and memorizing of emotions [93].

Studies using [3H]-GR 113808 in the rat have revealed that during development, prenatal expression is low, with the exception of the brainstem, indicating that 5-HT$_4$R is largely dispensable in development. Interestingly, the synchronous appearance of 5-HT$_4$ receptors and cholinergic markers validates the notion of 5-HT$_4$R-mediated control over acetylcholine release [94].

With age, 5-HT$_4$R expression goes down as older humans present lower 5-HT$_4$R binding [93].

Table 2 assembles data on expression changes in disease or that are pharmacologically induced.
### Table 1. Compilation of studies of expression of the 5-HT4 receptor in human, mouse and rat brain in the basal/healthy state.

| species         | Tissue                                      | Cell Type                                           | Transcript/Protein       | Method                          | Reference                          |
|-----------------|---------------------------------------------|----------------------------------------------------|--------------------------|---------------------------------|------------------------------------|
| **human**       | caudate nucleus, putamen, nucleus accumbens, globus pallidus, substantia nigra. Lesser densities in hippocampus and cortex | protein                                            | [3H]-R116712 and [3H]-pruclapride binding | Bonaventure et al., 2000, [10]     |
|                 | caudate nucleus, putamen, nucleus accumbens, and in the hippocampal formation but not in globus pallidus and substantia nigra | mRNA                                               | in situ hybridization     | Bonaventure et al., 2000, [10]     |
|                 | caudate nucleus, putamen, nucleus accumbens, globus pallidus, substantia nigra, hippocampus (CA1, subiculum), neocortex | protein                                            | [125I]-SB 20771 binding  | Varnas et al., 2003, [92],         |
| human, calf, guinea pig | caudate nucleus, lenticular nucleus, substantia nigra, hippocampus, frontal cortex | protein                                            | [3H]-GR 113808 binding to membrane preparations | Domenech et al., 1994, [82]         |
|                 | caudate nucleus, lateral pallidum, putamen, medial pallidum, temporal cortex, hippocampus, amygdala, frontal cortex, cerebellar cortex | mRNA, protein                                      |                         | Vilaró et al., 1996, [65]          |
| **rat**         | caudate putamen, ventral striatum, medial habenula and hippocampus | mRNA                                               | in situ hybridization     | Vilaró et al., 1996, [65]          |
|                 | prefrontal cortex                           | 60% of pyramidal glutamatergic neurons             | mRNA, protein indirect through stimulation | Feng et al., 2001, [80]            |
|                 | basal forebrain, hippocampus, cortex        | GABAergic, glutamatergic and parvalbumin-containing neurons, hippocampal and cortical glutamatergic neurons | mRNA in situ hybridization | Penas-Cazorla et al., 2015, [78]    |
| **rat, guinea pig** | stratum, globus pallidus, hippocampus, substantia nigra, olfactory tubercle | protein                                            | [3H]-GR 113808 binding    | Grossman et al., 1993, [92]         |
|                 | striatum, hippocampus                        | striatal GABAergic projection neurons, projection from dentate granule cells to field CA3, habenulo-hippocampal pathway, somatodendritically and axonally | mRNA, protein [125I]-SB 207710 binding, in situ hybridization | Vilaró et al., 2005, [62]          |
| **mouse**       | stratum                                      | GABAergic projection neurons but not dopaminergic neurons | protein                  | Kainic acid lesions and [3H]-GR 113808 binding | Compan et al., 1996, [11]          |
|                 | striatum                                     | GABAergic projection neurons                       | protein                  | immunohistochemistry             | Egeland et al., 2011, [59]         |
|                 | dentate gyrus                                | mature granule cells                               | protein                  | LacZ-IR staining                 | Iimoto et al., 2015, [90]          |
### Table 2. Compilation of studies of expression of the 5-HT4 receptor in human, mouse and rat brain in the disease state, in disease models or after drug treatment.

| Species | Tissue | Condition/Treatment | Direction of Change | Transcript/Protein | Method | Reference |
|---------|--------|---------------------|---------------------|--------------------|--------|-----------|
| human   | frontal cortex, caudate nucleus | suicide victims | up | protein | antagonist binding | Rosel et al., 2004, [96] |
|         | hippocampus, frontal cortex | association with bipolar disorder | | SNP's | sequencing of PCR products | Ohtsuki et al., 2002, [97] |
| putamen | Huntington’s disease | down | protein | [3H]-GR113808 binding | Reynolds et al., 1995, [95] |
| hippocampus | cognition, episodic memory, recall | negative correlation | protein | PET, [11C]-SB207145 | Haahr et al., 2013, [98] |
| nucleus accumbens, ventral pallidum, orbitofrontal cortex, hippocampus | body mass index, obesity | positive correlation | protein | PET, [11C]-SB207145 | Haahr et al., 2012, [99] |
| rat     | striatum, subthalamic nucleus, hippocampus | lesion of serotonergic nuclei | up in rostral dorsal, ventral striatum, substantia nigra, hippocampus | protein | [3H]-GR113808 binding | Compan et al., 1996, [11] |
|        | striatum (caudate putamen, globus pallidus) | lesion of DA neurons | up | protein | [3H]-GR113808 binding | Compan et al., 1996, [11] |
|        | hippocampus, lateral globus pallidus | lesion of DA neurons | down | protein | [3H]-SB207145 binding | Licht et al., 2009, [100] |
|        | hippocampus, hypothalamus, caudate putamen, nucleus accumbens, Globus pallidus | 21 days paroxetine (SSRI) | down after SSRI | protein | [3H]-SB207145 binding | Licht et al., 2009, [100] |
|        | hippocampus, hypothalamus | 4 days of 5-HT depletion | up after 5-HT depletion | protein | [3H]-SB207145 binding | Licht et al., 2009, [100] |
|        | hippocampus (CA1), striatum | 21 days of fluoxetine (SSRI) | down | protein | [3H]-GR113808 binding | Vidal et al., 2009, [101] |
|        | 15 regions incl. hippocampus | learning: autoshaping test for food retrieval | upregulated in most regions | protein | [3H]-GR113808 binding | Manuel-Apolinar et al., 2005, [102] |
|        | caudate putamen, nucleus accumbens | rat models of obesity | up | protein | [3H]-GR113808 binding | Ratner et al., 2012, [103] |
|        | hippocampus | maternal deprivation, unpredictable stress | down | mRNA, protein | qPCR and Western blotting | Bai et al., 2014, [51] |
| mouse   | striatum | 6-OHDA lesion model of Parkinson’s disease | down in D2 MSNs | mRNA | Affymetrix GeneChip microarray | Heiman et al., 2014, [104] |
|         | ventral hippocampus | bulbectomy | up in ventral hippocampus, down in olfactory tubercles | protein | [3H]-SB207145 binding | Licht et al., 2010, [97] |
|         | caudal putamen | GR (+/−) mice | up | protein | [3H]-SB207145 binding | Licht et al., 2010, [97] |
|         | midbrain raphe nuclei and VTA | social defeat | up after defeat | mRNA | RNA seq | Kudryavtseva et al., 2017, [105] |
|         | prefrontal cortex | restraint stress | up after restraint | mRNA | qPCR | Jean et al., 2017, [22] |
7.1. Depression and Anxiety

The understanding of the roles that 5-HT$_4$ receptors play in mood disorders mainly comes from preclinical studies. Several rodent models of depression and anxiety, such as bulbectomy, glucocorticoid receptor heterozygous mice, social defeat stress or exposure to prenatal stress, all indicated changes in 5-HT$_4$R expression: In mice, the experience of social defeat led to 5-HT$_4$R mRNA up-regulation in the midbrain raphe nuclei and the VTA, as determined by RNA seq [105]. Similarly, restraint stress induced hypophagia and increased 5-HT$_4$R mRNA levels in the medial prefrontal cortex [22]. In contrast, maternal stress led to a reduction of all mouse 5-HT$_4$R variants on the mRNA level as assessed by qPCR, with the strongest difference observed for the (b) variant, while chemically induced 5-HT depletion in the embryo only affected the expression of the (b) variant in the embryonic telencephalon [106].

After bulbectomy, 5-HT$_4$R protein binding was increased in the rat ventral hippocampus and olfactory tubercles but unchanged in the dorsal hippocampus, frontal and caudal caudate putamen. 5-HT transporter (SERT) binding was unchanged in the hippocampus and caudate putamen and slightly down in lateral septum and globus pallidus [97]. GR(+/−) mice had increased 5-HT$_4$R binding in the caudal caudate putamen and the olfactory tubercles, decreased SERT binding in the frontal caudate putamen but no changes for 5-HT$_4$R and SERT in the hippocampus [97]. In contrast, in the Flinders Sensitive Line, a rat model of depression, 5-HT$_4$R binding was decreased in the dorsal and ventral hippocampus [100].

A 3-week long treatment regimen with the SSRI fluoxetine decreased the density of 5-HT$_4$ receptor binding in the CA1 field of hippocampus as well as in several areas of the striatum in rats [101]. In contrast, 5-HT$_4$R in layer 5 of the cerebral cortex was shown to be selectively upregulated after fluoxetine treatment in p11-GFP bacTRAP mice [107]. Interestingly, when 5-HT$_4$R expression was quantified by qPCR on whole cortical lysate no difference in response to fluoxetine treatment was detected, while a 16-fold upregulation in the deep cortical layers was found after TRAP purification. This study clearly demonstrates that methods of purification and enrichment are necessary to achieve a resolution that is sufficient to characterize the dynamics of 5-HT$_4$R expression. Given that chronic fluoxetine in mice lead to a specific upregulation in layer 5 of the cortex [107], it is clear that research into expression changes needs to be approached with techniques achieving high resolution since global expression changes might be counterweighed by cell-type and subregion-specific compensatory changes.

Data generated in humans with $[^{11}]$C-SB 207145 brain PET imaging suggest that 5-HT$_4$R is involved in the neurobiological mechanism underlying familial risk for depression, and that lower striatal but not cortical 5-HT$_4$ receptor binding is associated with an increased risk for developing major depressive disorder [108]. However, in the caudate nucleus, the relationship between 5-HT$_4$R and suicide risk was inverse: Postmortem studies found increased 5-HT$_4$ receptor binding in the caudate nucleus and frontal cortex of depressed suicide victims [96]. Polymorphisms of the htr4 gene were found to correlate with major depression and/or bipolar disorders [109].

A PET study showed a global reduction in cerebral 5-HT$_4$R binding in healthy volunteers after a 3 week treatment with fluoxetine [110], pointing towards an inverse correlation of global 5-HT$_4$R binding and synaptic serotonin levels, or an activity-induced downregulation response.

In summary, there is strong evidence regarding the involvement of 5-HT$_4$R in the etiology and expression of depression; however, different preclinical models of depression and anxiety and binding studies in humans show different responses in 5-HT$_4$R expression in different brain regions that need to be further addressed.

7.2. Food Intake and Obesity

High levels of 5-HT$_4$R are observed in obese humans [99] and in overfed rats in the caudate putamen and the nucleus accumbens shell [103]. Injection of 5-HT$_4$R agonist into the nucleus accumbens reduces the drive to eat while injection of 5-HT$_4$R antagonist or knockdown in the
nucleus accumbens induces hyperphagia in fed mice [111]. These data suggest that changes in 5-HT$_4$R expression may play a role in eating disorders. Indeed, PET studies showed a correlation between the body mass index and 5-HT$_4$R protein in the nucleus accumbens, ventral pallidum, the orbitofrontal cortex and hippocampus [99]. Furthermore, the density of 5-HT$_4$ receptors was found to be decreased in the temporal cortex of Alzheimer’s disease patients who also suffer from hyperphagia [112].

7.3. Memory and Alzheimer’s Disease

A role for 5-HT$_4$R in Alzheimer’s disease has been described: The receptor was linked to APP processing and β-amyloid generation in rodent models of Alzheimer’s disease. Chronic administration of 5-HT$_4$R agonists reduced β-amyloid pathology through the promotion of non-amyloidogenic cleavage of the precursor of Aβ and the consequent promotion of the neurotrophic protein, sAPPα, thereby alleviating AD pathology as well as reducing plaque load [113,114]. In a transgenic Alzheimer’s mouse model, stimulation of 5-HT$_4$R exerted pro-cognitive effects, which resulted in enhanced learning through increasing acetylcholine levels [24,113,115,116]. This body of work is largely based on the use of 5-HT$_4$R pharmacological tools and shows that 5-HT$_4$R stimulation enhanced performance on memory tasks in rodents while receptor antagonists induced worsening of the performance on these tasks.

During memory consolidation in a food retrieval learning paradigm, 5-HT$_4$ radioligand binding showed an upregulation in olfactory lobule, caudate putamen, fundus striatum, hippocampus (CA2) and several cortical regions of young adult animals. In contrast, some but not all tested regions of older rats (hippocampal CA2 and CA3 areas, and frontal, parietal, and temporal cortex) expressed reduced 5-HT$_4$ receptor density [102] pointing towards age-dependent regulation of 5-HT$_4$R expression.

In humans, PET studies with [11C]-SB207145 as tracer and an episodic memory verbal learning test, resulted in an unexpected negative correlation of 5-HT$_4$R and memory function in healthy young volunteers. Thus, in humans, unlike what was hypothesized based on rodent studies, fewer hippocampal 5-HT$_4$Rs are representative of a better episodic memory function [98]. In newly diagnosed Alzheimer’s disease patients, 5-HT$_4$R binding was positively correlated to β-amyloid burden and negatively to cognitive performance (MMSE score) suggesting that cerebral 5-HT$_4$R is upregulated during preclinical stage, possibly as compensatory effect to decreased levels of interstitial 5-HT [117].

No preclinical studies exist to date that show changes in 5-HT$_4$R expression in mouse models of Alzheimer’s disease. In humans, [3H]-GR 113808 labeling of post mortem brain tissue showed decreased 5-HT$_4$-receptor expression in the hippocampus and prefrontal cortex in patients with Alzheimer’s disease [95]. However, another study contradicts these findings revealing no changes in 5-HT$_4$R density in Alzheimer’s disease in frontal and temporal cortices [118].

Thus, to corroborate the relation between 5-HT$_4$R expression and memory function in humans, in healthy and disease states, further studies are warranted.

7.4. Schizophrenia

Limited evidence indicates that 5-HT$_4$R polymorphisms could predispose to schizophrenia [119] and attention deficit hyperactivity disorder (ADHD) [120].

7.5. Parkinson’s Disease

Expression of 5-HT$_4$R was found to be altered in rodent models of PD. Depletion of dopamine neurons by 6-OHDA leads to increased 5-HT$_4$R receptor binding in the caudal caudate-putamen and globus pallidus (+93%) [11]. In contrast, in 6-OHDA lesioned mice, 5-HT$_4$R mRNA was reduced (4-fold) while L-DOPA treatment doubled the 5-HT$_4$R expression in the D2-SPNs. In D1-SPNs, changes only occurred after L-DOPA treatment (2-fold) [104]. For technical reasons in this study, no comparison of the total expression levels in D1- and D2-SPNs could be made. However, these findings are very interesting since they suggest a potential role for 5-HT$_4$R in L-DOPA induced dyskinesia. In post-mortem studies of PD subjects, 5-HT$_4$R binding in putamen and substantia nigra was found to be unaltered [91].
The small number of patients (N = 6), and the non-discrimination of medication, treatment duration and disease severity does, in our opinion, not allow a conclusive statement.

Future work involving spatially restricted deletions of 5-HT\textsubscript{4} receptors or local administration of pharmacological ligands is necessary to more precisely determine the cellular and circuit-based mechanisms by which 5-HT\textsubscript{4} receptors influence behavior.

8. Other Proteins Affecting 5-HT\textsubscript{4}R Signaling

8.1. SERT (5-HTT)

It is not surprising that genetic alteration of the serotonin transporter gene (5-HTT) has implications in mood disorders: For example, mice overexpressing SERT (OE) or with SERT deletion (KO) present anxiolytic-like or more anxious behaviors, respectively, when compared to WT littermates [121,122]. At the molecular level, in the homozygous SERT KO mice, the activity of the 5-HT\textsubscript{1A} autoreceptor is decreased [123,124] while 5-HT\textsubscript{2A} receptor function is enhanced [125–127]. Protein levels of 5-HT\textsubscript{4}R are altered in the SERT KO and SERT OE mice. Precisely, autoradiography studies with [3H]-SB 207145 radioligand show increased 5-HT\textsubscript{4}R receptor binding in the SERT OE mice in all brain regions but the amygdala. Inversely, in the SERT KO mice, 5-HT\textsubscript{4}R binding is decreased in all regions studied. This is consistent with studies providing evidence that chronic treatment with SSRIs in healthy individuals decreased 5-HT\textsubscript{4}R binding as seen in PET imaging [110]. Studies in rodents replicate this result of decreased 5-HT\textsubscript{4}R-dependent activation of adenylate cyclase and reduced electrophysiological activity in the hippocampus [128]. In a similar fashion, mice overexpressing 5-HT\textsubscript{4}R in the mPFC exhibit stress-induced hypophagia and a corresponding 5-HT\textsubscript{4}R-dependent downregulation of SERT and 5-HT\textsubscript{1A} transcripts. Oppositely, siRNA mediated knockdown of 5-HT\textsubscript{4}R in the mPFC induces hyperphagia [22].

These studies are important because they highlight that altered 5-HT concentration is most likely responsible for changes in 5-HT\textsubscript{4}R receptor binding as a compensatory mechanism; they also highlight the bi-directionality of this process, since exogenous alterations in 5-HT\textsubscript{4}R levels induce changes in 5-HT availability, negatively regulating the expression of SERT as well as serotonin receptors.

8.2. Adaptor Protein p11

S100 calcium effector protein p11 (S100A10), a depression marker protein, has been identified in a yeast-based screening system as a binding partner to 5-HT\textsubscript{4}R, with greater affinity to 5-HT\textsubscript{4}R than to other serotonin receptors, such as 5-HT\textsubscript{1B} and 5-HT\textsubscript{1D} receptors [129]. p11 co-localizes with 5-HT\textsubscript{4}R in brain regions that play an important role in major depressive disorder like cingulate cortex, hippocampus, amygdala and striatum as seen by in situ hybridization and immunohistochemistry using the transgenic bac-GFP mice where GFP is expressed under the 5-HT\textsubscript{4}R promoter. p11 KO mice show reduced 5-HT\textsubscript{4}R protein in radioligand binding assays, are behaviorally less sensitive to antidepressant treatment and do not respond to 5-HT\textsubscript{4}R agonist. As binding partner of 5-HT\textsubscript{4}R and adaptor protein for many other GPCRs, p11 recruits 5-HT\textsubscript{4}R to the site of its action, the plasma membrane [129].

8.3. CK2

CK2 is a constitutively active and ubiquitously expressed kinase. Recently, CK2 has been identified as a negative regulator of the 5-HT\textsubscript{4}R [46]. Knockdown or inhibition of CK2 in vitro elevates 5-HT\textsubscript{4}R receptor-dependent cAMP generation and increases receptor localization at the plasma membrane in monkey COS7 cells. Interestingly, in the mouse brain, mRNA upregulation of the 5-HT\textsubscript{4}R is specific to the PFC. Virally-mediated focal knockdown of CK2 or overexpression of 5-HT\textsubscript{4}R in the mPFC generates an anti-depressed and anxiolytic-like phenotype that is similar to the phenotypes observed with CK2 knockout in the forebrain driven by Emx1-Cre or Drd1a-Cre. In addition, such conditional CK2 KO mice are more responsive to antidepressant drugs and 5-HT\textsubscript{4}R agonist (RS 67333) treatment [46].
8.4. Testosterone

Several studies describe the relationship between sex hormones and serotonin in mood-related disorders. The prevalence of major depressive disorder is 1.7 times higher in women than in men [130]. Several studies correlate depressive episodes with hormonal changes especially in the menstrual cycle in women although the exact mechanism by which this happens is not clear [130]. In men, it has been found that plasma testosterone negatively correlates with brain 5-HT₄R binding in humans throughout the brain [131]. Higher levels of testosterone lead to increased serotonergic signaling but whether testosterone directly regulates levels through steroid hormone receptors co-localized with 5-HT₄R or by an indirect mechanism (e.g., increased of serotonergic tonus through other targets) to decrease expression of 5-HT₄R needs to be further examined.

8.5. Nav1.7

Nav1.7 is a voltage-gated sodium channel required for nociceptive neuronal activation. While humans lacking Nav1.7 and genetic KO mice show absence of pain, a pharmacological antagonist of this channel failed to decrease pain sensitivity, indicating that receptor signaling mediated activation of nociceptive neurons might not be the only mechanism involved in pain alleviation. For example, loss of Nav1.7 coincides with upregulation of met-enkephalin, an endogenous opioid peptide in sensory neurons, increasing opioid activity and anti-nociceptive signaling. In addition, Nav 1.7 KO mice present reduced levels of 5-HT₄R in dorsal root ganglia [132]. Both effects, i.e., changes in enkephalin and 5-HT₄R expression and signaling, take place in peripheral nociceptive neurons and together contribute to the analgesic effect [133].

9. Conclusions

It is clear that changes in 5-HT₄R expression correlate with several disease states. In order to clarify whether these changes are also causative or involved in the etiology of disease, the expression needs to be assessed on a cellular level in preclinical models. While 5-HT₄R overexpression in rodents, for example, through virus injection, is truly helpful in delineating the role of 5-HT₄R in certain brain regions and cell types, these experiments have the disadvantage of introducing the gene under an exogenous promoter thus leading to non-physiological levels of expression and lacking the opportunity to study transcriptional regulation. Thus, it is preferable to study transgenic mice in which a labeled version of the receptor is expressed under its endogenous promoter such as the transgenic mouse line where the β-galactosidase gene is knocked in at the htr4 gene locus [90], enabling unambiguous cell identification or cell-type specific purifications and quantification methods. Human PET or post mortem studies are important to verify hypotheses but may not allow the resolution needed.

Another aspect that has to be taken into consideration is the fact that splicing variants differ between species. The factors responsible for these differences are unknown but may be important in understanding human pathologies. To bridge this knowledge gap, it would be interesting to generate, through streamlining the gene architecture by engineering/deleting of splicing sites, mice which expressing specific (human) variants only and to determine whether this will affect 5-HT₄R-dependent phenotypes (e.g., electrophysiological properties, neurotransmitter release, receptor homeostasis, behavior and biochemical signaling cascades). Once this has been established, we will be in a better position to develop more suitable 5-HT₄R mouse models to study human disease.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Palacios, J.M. Serotonin receptors in brain revisited. Brain Res 1645. [CrossRef] [PubMed]
2. Riad, M.; Garcia, S.; Watkins, K.C.; Jodoin, N.; Doucet, E.; Langlois, X.; el Mestikawey, S.; Hamon, M.; Descarrries, L. Somatodendritic localization of 5ht1a and preterminal axonal localization of 5-htr1b serotonin receptors in adult rat brain. J. Comp. Neurol. 2000, 417, 181–194. [CrossRef]
1. Vollenweider, F.X.; Kometer, M. The neurobiology of psychedelic drugs: Implications for the treatment of mood disorders. *Nat. Rev. Neurosci.* 2010, 11, 642–651. [CrossRef] [PubMed]

2. Compan, V.; Daszuta, A.; Salin, P.; Sebben, M.; Bockaert, J.; Dumuis, A. Lesion study of the distribution of serotonin 5-htr4 receptors in rat basal ganglia and hippocampus. *Eur. J. Neurosci.* 1996, 8, 2591–2598. [CrossRef] [PubMed]

3. Callahan, M.J. Irritable bowel syndrome neuropharmacology. A review of approved and investigational compounds. *J. Clin. Gastroenterol.* 2002, 35, S58–S67. [CrossRef] [PubMed]

4. Tecott, L.H.; Sun, L.M.; Akana, S.F.; Strack, A.M.; Lowenstein, D.H.; Dallman, M.F.; Julius, D. Eating disorder and epilepsy in mice lacking 5-htr2c serotonin receptors. *Nature* 1995, 374, 542–546. [CrossRef] [PubMed]

5. Vollenweider, F.X.; Kometer, M. The neurobiology of psychedelic drugs: Implications for the treatment of mood disorders. *Nat. Rev. Neurosci.* 2010, 11, 642–651. [CrossRef] [PubMed]

6. Maillet, M.; Gastineau, M.; Bochet, P.; Asselin-Labat, M.L.; Morel, E.; Laverriere, J.N.; Lompre, A.M.; Compan, V.; Zhou, M.; Grailhe, R.; Gazzara, R.A.; Martin, R.; Gingrich, J.; Dumuis, A.; Brunner, D.; Bockaert, J.; Hen, R. Attenuated response to stress and novelty and hypersensitivity to seizures in 5-htr4 receptor knock-out mice. *J. Neurosci.* 2004, 24, 412–419. [CrossRef] [PubMed]
22. Jean, A.; Laurent, L.; Delaunay, S.; Doly, S.; Dusticier, N.; Linden, D.; Neve, R.; Maroteaux, L.; Nieoullon, A.; Compan, V. Adaptive control of dorsal raphe by 5-HT4 in the prefrontal cortex prevents persistent hypophagia following stress. *Cell Rep.* 2017, 21, 901–909. [CrossRef] [PubMed]

23. Jean, A.; Laurent, L.; Bockaert, J.; Charnay, Y.; Dusticier, N.; Nieoullon, A.; Barrot, M.; Neve, R.; Compan, V. The nucleus accumbens 5-htr(4)-cart pathway ties anorexia to hyperactivity. *Transl. Psychiatry* 2012, 2, e203. [CrossRef] [PubMed]

24. Consolo, S.; Arnaboldi, S.; Giorgi, S.; Russi, G.; Ladinsky, H. 5-HT4 receptor stimulation facilitates acetylcholine release in rat frontal cortex. *Neuroreport* 1994, 5, 1230–1232. [CrossRef] [PubMed]

25. Siniscalchi, A.; Badini, I.; Beani, L.; Bianchi, C. 5-HT4 receptor modulation of acetylcholine outflow in guinea pig brain slices. *Neuroreport* 1999, 10, 547–551. [CrossRef] [PubMed]

26. Bijak, M.; Misgeld, U. Effects of serotonin through serotonin1a and serotonin4 receptors on inhibition in the guinea-pig dentate gyrus in vitro. *Neuroscience* 1997, 78, 1017–1026. [CrossRef]

27. Meneses, A.; Hong, E. Effects of 5-HT4 receptor agonists and antagonists in learning. *Pharmacol. Biochem. Behav.* 1997, 56, 347–351. [CrossRef]

28. Fontana, D.J.; Daniels, S.E.; Wong, E.H.; Clark, R.D.; Eglen, R.M. The effects of novel, selective 5-hydroxytryptamine (5-HT4) receptor ligands in rat spatial navigation. *Neuropharmacology* 1997, 36, 689–696. [CrossRef]

29. Mohler, E.G.; 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. [CrossRef] [PubMed]

30. Quiedeville, A.; Bouloard, M.; Hamidouche, K.; Da Silva Costa-Aze, V.; Nee, G.; Rochais, C.; Dallemagne, P.; Fabis, F.; Freret, T.; Bouet, V. Chronic activation of 5-HT4 receptors or blockade of 5-HT6 receptors improve memory performances. *Behav. Brain Res.* 2015, 293, 10–17. [CrossRef] [PubMed]

31. Marchetti, E.; Jacquet, M.; Escoffier, G.; Bartolini, A.; Dumuis, A.; Bockaert, J.; Roman, F.S. Enhancement of reference memory in aged rats by specific activation of 5-HT(4) receptors using an olfactory associative discrimination task. *Brain Res.* 2011, 1405, 49–56. [CrossRef] [PubMed]

32. Letty, S.; Child, R.; Dumuis, A.; Pantaloni, A.; Bockaert, J.; Rondouin, G. 5-HT4 receptors improve social olfactory memory in the rat. *Neuropharmacology* 1999, 37, 681–687. [CrossRef]

33. Lelong, V.; Dauphin, F.; Bouloard, M. Rs 67333 and d-cycloserine accelerate learning acquisition in the rat. *Neuropharmacology* 2001, 41, 517–522. [CrossRef]

34. Lamirault, L.; Simon, H. Enhancement of place and object recognition memory in young adult and old rats by rs 67333, a partial agonist of 5-HT4 receptors. *Neuropharmacology* 2001, 41, 844–853. [CrossRef]

35. Marchetti, E.; Dumuis, A.; Bockaert, J.; Soumireu-Mourat, B.; Roman, F.S. Differential modulation of the 5-htr(4) receptor agonists and antagonists on rat learning and memory. *Neuropharmacology* 2000, 39, 2017–2027. [CrossRef]

36. Galeotti, N.; Ghelardini, C.; Bartolini, A. Role of 5-HT4 receptors in the mouse passive avoidance test. *J. Pharmacol. Exp. Ther.* 1998, 286, 1115–1121. [PubMed]

37. 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. [CrossRef] [PubMed]

38. Chen, L.; Yung, K.K.; Chan, Y.S.; Yung, W.H. 5-HT excites globus pallidus neurons by multiple receptor mechanisms. *Neuroscience* 2008, 151, 439–451. [CrossRef] [PubMed]

39. Bianchi, C.; Rodi, D.; Marino, S.; Beani, L.; Siniscalchi, A. Dual effects of 5-HT(4) receptor activation on gaba release from guinea pig hippocampal slices. *Neuroreport* 2002, 13, 2177–2180. [CrossRef] [PubMed]

40. Bonhomme, N.; De Deurwaerdere, P.; Le Moal, M.; Spampinato, U. Evidence for 5-HT4 receptor subtype involvement in the enhancement of striatal dopamine release induced by serotonin: A microdialysis study in the halothane-anesthetized rat. *Neuropsychopharmacology* 1995, 34, 269–279. [CrossRef] [PubMed]
42. Navailles, S.; Di Giovanni, G.; De Deurvaerdere, P. The 5-htr4 agonist prucalopride stimulates l-dopa-induced dopamine release in restricted brain regions of the hemiparkinsonian rat in vivo. *CNS Neurosci. Ther.* 2015, 21, 745–747. [CrossRef] [PubMed]

43. Conductier, G.; Dusticiér, 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-htr4 receptor knock-out mouse. *Eur. J. Neurosci.* 2006, 24, 1053–1062. [CrossRef] [PubMed]

44. Lucas, G.; Compan, V.; Charnay, Y.; Neve, R.L.; Nestler, E.J.; Bockaert, J.; Barrot, M.; Debonnel, G. Frontocortical 5-htr4 receptors exert positive feedback on serotonergic activity: Viral transfections, subacute and chronic treatments with 5-htr4 agonists. *Biol. Psychiatry* 2005, 57, 918–925. [CrossRef] [PubMed]

45. Covington, H.E., 3rd; Lobo, M.K.; Maze, I.; Vialou, V.; Hyman, J.M.; Zaman, S.; LaPlant, Q.; Mouzon, E.; House, J.S.; Li, H.; DeGraff, L.M.; Flake, G.; Zeldin, D.C.; London, S.J. Genetic variation in htr4 and lung function. *Hepatology* 2014, 60, 16082–16090. [CrossRef] [PubMed]

46. Castello, J.; LeFrancois, B.; Flajolet, M.; Greengard, P.; Friedman, E.; Rebholz, H. Ck2 regulates 5-ht4 receptor signaling and modulates depressive-like behavior. *Mol. Psychiatry* 2018, 23, 872–882. [CrossRef] [PubMed]

47. Kennett, G.A.; Bright, F.; Trail, B.; Blackman, T.P.; Sanger, G.J. Anxiolytic-like actions of the selective 5-htr4 receptor antagonists sb 204070a and sb 207266a in rats. *Neuropharmacology* 1997, 36, 707–712. [CrossRef] [PubMed]

48. Lucas, G.; Rymar, V.V.; Du, J.; Mnie-Filali, O.; Bisgaard, C.; Manta, S.; Lambas-Senas, L.; Wiborg, O.; Haddjeri, N.; Pineyro, G.; et al. Serotonin(4) (5-htr4) receptor agonists are putative antidepressants with a rapid onset of action. *Neuron* 2007, 55, 712–725. [CrossRef] [PubMed]

49. Hiroi, T.; Hayashi-Kobayashi, N.; Nagumo, S.; Ino, M.; Okawa, Y.; Aoba, A.; Matsui, H. Identification and characterization of the human serotonin-4 receptor gene promoter. *Biochem. Biophys. Res. Commun.* 2001, 289, 337–344. [CrossRef] [PubMed]

50. Wohlfarth, C.; Schmitteckert, S.; Hartle, J.D.; Houghton, L.A.; Dweep, H.; Fortea, M.; Assadi, G.; Braun, A.; Mederer, T.; Pohner, S.; et al. Mir-16 and mir-103 impact 5-ht4 receptor signalling and correlate with symptom profile in irritable bowel syndrome. *Sci. Rep.* 2017, 7, 14680. [CrossRef] [PubMed]

51. Bai, M.; Zhu, X.Z.; Zhang, Y.; Zhang, S.; Zhang, L.; Xue, L.; Zhong, M.; Zhang, X. Anhedonia was associated with the dysregulation of hippocampal htr4 and microrna let-7a in rats. *Neurosci. Ther.* 2018, 35, 129, 135–141. [CrossRef] [PubMed]

52. Hancock, D.B.; Eijgelsheim, M.; Wilk, J.B.; Gharib, S.A.; Loehr, L.R.; Marcianite, K.D.; Franceschini, N.; van Durme, Y.M.; Chen, T.H.; Barr, R.G.; et al. Meta-analyses of genome-wide association studies identify multiple loci associated with pulmonary function. *Nat. Genet.* 2010, 42, 45–52. [CrossRef] [PubMed]

53. Soler Artigas, M.; Loth, D.W.; Wain, L.V.; Gharib, S.A.; Obeidat, M.; Tang, W.; Zhao, J.H.; Smith, A.V.; Heckbert, S.R.; Luyten, W. Structure of the human serotonin 5-ht4 receptor gene and cloning of a novel 5-ht4 splice variant. *Eur. J. Pharmacol.* 2004, 1082–1090. [CrossRef] [PubMed]

54. Wilk, J.B.; Shrine, N.R.; Loehr, L.R.; Zhao, J.H.; Manichaikul, A.; Lopez, L.M.; Smith, A.V.; Heckbert, S.R.; Smolonska, J.; Tang, W.; et al. Genome-wide association studies identify chrna5/3 and htr4 in multiple loci associated with pulmonary function. *Nat. Genet.* 2010, 42, 55, 57, 604. [CrossRef] [PubMed]

55. Blom, J.; Klaver, C.C.; van den Heuvel, I.; Willens, J.; Cremers, K.; et al. Long-term treatment with prucalopride restores the 5-ht4 receptor signaling and ameliorates motor performance in a mouse model for ross syndrome. *Brain Behav.* 2011, 1, 125, 595–597. [CrossRef] [PubMed]

56. Brattelid, T.; Kvingedal, A.M.; Krobert, K.A.; Andressen, K.W.; Bach, T.; Hystad, M.E.; Kaumann, A.J.; Bendz, K.; Valberg, M.; et al. 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. [CrossRef] [PubMed]

57. Coupar, I.M.; Desmond, P.V.; Irving, H.R. Human 5-htr4 and 5-htr7 receptor splice variants: Are they important? *Curr. Neuropharmacol.* 2007, 5, 224–231. [CrossRef] [PubMed]

58. Rady, A.M.; Kelsell, R.E.; Houp, J.A.; Kelly, F.M.; Medhurst, A.D.; Cox, H.M.; Calver, A.R. Identification of a novel 5-htr4 receptor splice variant (r5-htr4c1) and preliminary characterisation of specific 5-htr4a and 5-htr4b receptor antibodies. *Eur. J. Pharmacol.* 2009, 604, 1–11. [CrossRef] [PubMed]

59. Bender, E.; Pindon, A.; van Oers, I.; Zhang, Y.B.; Gommers, W.; Verhasselt, P.; Jurzak, M.; Leysen, J.; Luyten, W. Structure of the human serotonin 5-htr4 receptor gene and cloning of a novel 5-htr4 splice variant. *J. Neurochem.* 2000, 74, 478–489. [CrossRef] [PubMed]
61. Vilaro, M.T.; Domenech, T.; Palacios, J.M.; Mengod, G. Cloning and characterization of a novel human 5-ht4 receptor variant that lacks the alternatively spliced carboxy terminal exon. RT-PCR distribution in human brain and periphery of multiple 5-ht4 receptor variants. *Neuropsychopharmacology* 2002, 42, 60–73. [CrossRef] [PubMed]

62. Vilaro, M.T.; 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. [CrossRef] [PubMed]

63. Claeysen, S.; Faye, P.; Sebben, M.; Taviaux, S.; Bockaert, J.; Dumuis, A. 5-ht4 receptors: Cloning and expression of new splice variants. *Ann. N. Y. Acad. Sci.* 1998, 861, 49–56. [CrossRef] [PubMed]

64. Kaizuka, T.; Hayashi, T. Comparative analysis of palmitoylation sites of serotonin (5-ht) receptors in vertebrates. *Neuropsychopharmacol. Rep.* 2018, 38, 75–85. [CrossRef] [PubMed]

65. Vilaro, M.T.; Cortes, R.; Gerald, C.; Branchek, T.A.; Palacios, J.M.; Mengod, G. Localization of 5-ht4 receptor mRNA in rat brain by in situ hybridization histochemistry. *Mol. Brain Res.* 1996, 43, 356–360. [CrossRef] [PubMed]

66. Claeysen, S.; Sebben, M.; Becamel, C.; Bockaert, J.; Dumuis, A. Novel brain-specific 5-ht4 receptor splice variants show marked constitutive activity: Role of the c-terminal intracellular domain. *Mol. Pharmacol.* 1999, 55, 910–920. [PubMed]

67. 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-ht4 receptor splice variant: Roles in receptor targeting. *J. Cell Sci.* 2004, 117, 5367–5379. [CrossRef] [PubMed]

68. Pindon, A.; Van Hecke, G.; Josson, K.; Van Gompel, P.; Lesage, A.; Leysen, J.E.; Jurzak, M. Internalization of human 5-ht4a and 5-ht4b receptors is splice variant dependent. *Biosci. Rep.* 2004, 24, 215–223. [CrossRef] [PubMed]

69. Pindon, A.; van Hecke, G.; van Gompel, P.; Lesage, A.S.; Leysen, J.E.; Jurzak, M. Differences in signal transduction of two 5-ht4 receptor splice variants: Compound specificity and dual coupling with galphas- and galphai/o-proteins. *Mol. Pharmacol.* 2002, 61, 85–96. [CrossRef] [PubMed]

70. Benovic, J.L.; Strasser, R.H.; Caron, M.G.; Lefkowitz, R.J. Beta-adrenergic receptor kinase: Identification of a novel protein kinase that phosphorylates the agonist-occupied form of the receptor. *Proc. Natl. Acad. Sci. USA* 1986, 83, 2797–2801. [CrossRef] [PubMed]

71. Lohse, M.J.; Benovic, J.L.; Codina, J.; Caron, M.G.; Lefkowitz, R.J. Beta-arrestin: A protein that regulates beta-adrenergic receptor function. *Science* 1990, 248, 1547–1550. [CrossRef] [PubMed]

72. Krupnick, J.G.; Benovic, J.L. The role of receptor kinases and arrestins in G protein-coupled receptor regulation. *Annu. Rev. Pharmacol. Toxicol.* 1998, 38, 289–319. [CrossRef] [PubMed]

73. Stadel, J.M.; Strulovici, B.; Nambi, P.; Lavin, T.N.; Briggs, M.M.; Caron, M.G.; Lefkowitz, R.J. Desensitization of the beta-adrenergic receptor of frog erythrocytes. Recovery and characterization of the down-regulated receptors in sequestered vesicles. *J. Biol. Chem.* 1983, 258, 3032–3038. [PubMed]

74. Ferguson, S.S. Evolving concepts in G protein-coupled receptor endocytosis: The role in receptor desensitization and signaling. *Pharmacol. Rev.* 2001, 53, 1–24. [PubMed]

75. Willets, J.M.; Mistry, R.; Nahorski, S.R.; Challiss, R.A. Specificity of G protein-coupled receptor kinase 6-mediated phosphorylation and regulation of single-cell m3 muscarinic acetylcholine receptor signaling. *Mol. Pharmacol.* 2003, 64, 1059–1068. [CrossRef] [PubMed]

76. Salom, D.; Wang, B.; Dong, Z.; Sun, W.; Padayatti, P.; Jordan, S.; Salon, J.A.; Palczewski, K. Post-translational modifications of the serotonin type 4 receptor heterologously expressed in mouse rod cells. *Biochemistry* 2012, 51, 214–224. [CrossRef] [PubMed]

77. Barthet, G.; Carrat, G.; Cassier, E.; Barker, B.; Gaven, F.; Pillot, M.; Framery, B.; Pellissier, L.P.; Augier, J.; Kang, D.S.; et al. Beta-arrestin1 phosphorylation by grk5 regulates G protein-independent 5-ht4 receptor signalling. *EMBO J.* 2009, 28, 2706–2718. [CrossRef] [PubMed]

78. Ponimaskin, E.; Dumuis, A.; Gaven, F.; Barthet, G.; Heine, M.; Glebov, K.; Richter, D.W.; Oppermann, M. Palmitoylation of the 5-hydroxytryptamine4a receptor regulates receptor phosphorylation, desensitization, and beta-arrestin-mediated endocytosis. *Mol. Pharmacol.* 2005, 67, 1434–1443. [CrossRef] [PubMed]

79. Ponimaskin, E.G.; Heine, M.; Joubert, L.; Sebben, M.; Bickmeyer, U.; Richter, D.W.; Dumuis, A. The 5-hydroxytryptamine(4a) receptor is palmitoylated at two different sites, and acylation is critically involved in regulation of receptor constitutive activity. *J. Biol. Chem.* 2002, 277, 2534–2546. [CrossRef] [PubMed]
81. Pena-Cazorla, R.; Vilaro, M.T. Serotonin 5-ht4 receptors and forebrain cholinergic system: Receptor expression in identified cell populations. *Brain Struct. Funct.* 2015, 220, 3413–3434. [CrossRef] [PubMed]

82. Domenech, T.; Beleta, J.; Fernandez, A.G.; Gristwood, R.W.; Cruz Sanchez, F.; Tolosa, E.; Palacios, J.M. Identification and characterization of serotonin 5-ht4 receptor binding sites in human brain: Comparison with other mammalian species. *Mol. Brain Res.* 1994, 21, 176–180. [CrossRef]

83. Feng, J.; Cai, X.; Zhao, J.; Yan, Z. Serotonin receptors modulate gaba(a) receptor channels through activation of anchored protein kinase c in prefrontal cortical neurons. *J. Neurosci.* 2001, 21, 6502–6511. [CrossRef] [PubMed]

84. Roychowdhury, S.; Haas, H.; Anderson, E.G. 5-ht1a and 5-ht4 receptor colocalization on hippocampal pyramidal cells. *Neuropsychopharmacology* 1994, 33, 551–557. [CrossRef]

85. Grossman, C.J.; Kilpatrick, G.J.; Bunce, K.T. Development of a radioligand binding assay for 5-ht4 receptors in guinea-pig and rat brain. *Br. J. Pharmacol.* 1993, 109, 618–624. [CrossRef] [PubMed]

86. Waeber, C.; Sebben, M.; Grossman, C.; Javoy-Agid, F.; Bockaert, J.; Dumuis, A. [3h]-gr113808 labels 5-ht4 receptors in the human and guinea-pig brain. *Neuropeptides* 1993, 4, 1239–1242. [CrossRef] [PubMed]

87. Waeber, C.; Sebben, M.; Nieoullon, A.; Bockaert, J.; Dumuis, A. Regional distribution and ontogeny of 5-ht4 binding sites in rodent brain. *Neuropsychopharmacology* 1994, 33, 527–541. [CrossRef]

88. Mengod, G.; Vilaro, M.T.; Raurich, A.; Lopez-Gimenez, J.F.; Cortes, R.; Palacios, J.M. 5-ht receptors in mammalian brain: Receptor autoradiography and in situ hybridization studies of new ligands and newly identified receptors. *Histochem. J.* 1996, 28, 747–758. [CrossRef] [PubMed]

89. Egeland, M.; Warner-Schmidt, J.; Greengard, P.; Svenningsson, P. Co-expression of serotonin 5-ht(1b) and 5-ht(4) receptors in p11 containing cells in cerebral cortex, hippocampus, caudate-putamen and cerebellum. *Neuropsychopharmacology* 2011, 61, 442–450. [CrossRef] [PubMed]

90. Imoto, Y.; Kira, T.; Sukeno, M.; Nishitani, N.; Nagayasu, K.; Nakagawa, T.; Kaneko, S.; Kobayashi, K.; Segi-Nishida, E. Role of the 5-ht4 receptor in chronic fluoxetine treatment-induced neurogenic activity and granule cell dematuration in the dentate gyrus. *Mol. Brain* 2015, 8, 29. [CrossRef] [PubMed]

91. Wong, E.H.; Reynolds, G.P.; Bonhaus, D.W.; Hsu, S.; Eglen, R.M. Characterization of [3h]gr113808 binding to 5-ht4 receptors in brain tissues from patients with neurodegenerative disorders. *Behav. Brain Res.* 1996, 73, 249–252. [CrossRef]

92. Varnas, K.; Halldin, C.; Pike, V.W.; Hall, H. Distribution of 5-ht4 receptors in the postmortem human brain—an autoradiographic study using [125I]sb 207710. *Eur. Neuropsychopharmacol.* 2003, 13, 228–234. [CrossRef]

93. Madsen, K.; Haahr, M.T.; Marner, L.; Keller, S.H.; Baare, W.F.; Svarer, C.; Hasselbalch, S.G.; Knudsen, G.M. Age and sex effects on 5-ht(4) receptors in the human brain: A [(11)c]sb 207145 pet study. *J. Cereb. Blood Flow Metab.* 2011, 31, 1475–1481. [CrossRef] [PubMed]

94. Waeber, C.; Sebben, M.; Bockaert, J.; Dumuis, A. Regional distribution and ontogeny of 5-ht4 binding sites in rat brain. *Behav. Brain Res.* 1995, 73, 259–262. [CrossRef]

95. Reynolds, G.P.; Mason, S.L.; Meldrum, A.; De Keeler, S.; Parnes, H.; Eglen, R.M.; Wong, E.H. 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. [CrossRef] [PubMed]

96. Rosel, P.; Arranz, B.; Urretavizcaya, M.; Oros, M.; San, L.; Navarro, M.A. Altered 5-ht2a and 5-ht4 postsynaptic receptors and their intracellular signalling systems IP3 and camp in brains from depressed violent suicide victims. *Neuropsychobiology* 2004, 49, 189–195. [CrossRef] [PubMed]

97. Licht, C.L.; Marcussen, A.B.; Wegener, G.; Overstreet, D.H.; Aznar, S.; Knudsen, G.M. Changes in 5-ht4 receptor and 5-ht transporter binding in olfactory bulbectomized and glucocorticoid receptor heterozygous mice. *Neurochem. Int.* 2010, 56, 603–610. [CrossRef] [PubMed]

98. Haahr, M.E.; Fisher, P.; Holst, K.; Madsen, K.; Jensen, C.G.; Marner, L.; Lehel, S.; Baare, W.; Knudsen, G.; Hasselbalch, S. The 5-ht4 receptor levels in hippocampus correlates inversely with memory test performance in humans. *Hum. Brain Mapp.* 2013, 34, 3066–3074. [CrossRef] [PubMed]

99. Haahr, M.E.; Svendsen, P.M.; Madsen, K.; Marner, L.; Ratner, C.; Billings, N.; Baare, W.F.; Knudsen, G.M. Obesity is associated with high serotonin 4 receptor availability in the brain reward circuitry. *Neuroimage* 2012, 61, 884–888. [CrossRef] [PubMed]

100. Licht, C.L.; Marcussen, A.B.; Wegener, G.; Overstreet, D.H.; Aznar, S.; Knudsen, G.M. 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. [CrossRef] [PubMed]
101. Vidal, R.; Valdizan, E.M.; 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. [CrossRef] [PubMed]

102. Manuel-Apolinar, L.; Rocha, L.; Pascoe, D.; Castillo, E.; Castillo, C.; Meneses, A. Modifications of 5-ht4 receptor expression in rat brain during memory consolidation. Brain Res. 2005, 1042, 73–81. [CrossRef] [PubMed]

103. Ratnert, C.; Ettrup, A.; Bueter, M.; Haahr, M.E.; Compan, V.; le Roux, C.W.; Levin, B.; Hansen, H.H.; Knudsen, G.M. Cerebral markers of the serotonergic system in rat models of obesity and after roux-en-y gastric bypass. Obesity 2012, 20, 2133–2141. [CrossRef] [PubMed]

104. Heiman, M.; Heilbut, A.; Francardo, V.; Kulicke, R.; Fenster, R.J.; Kolaczyk, E.D.; Mesirov, J.P.; Surmeier, D.J.; Cenci, M.A.; Greengard, P. Molecular adaptations of striatal spiny projection neurons during levodopa-induced dyskinesia. Proc. Natl. Acad. Sci. USA 2014, 111, 4578–4583. [CrossRef] [PubMed]

105. Kudryavtseva, N.N.; Smagin, D.A.; Kovalenko, I.L.; Galyamina, A.G.; Vishnivetskaya, G.B.; Babenko, V.N.; Orlov, Y.L. Serotonergic genes in the development of anxiety/depression-like state and pathology of aggressive behavior in male mice: Rna-seq data. Mol. Biol. 2017, 51, 288–300. [CrossRef] [PubMed]

106. Chen, A.; Kelley, L.D.; Janusonis, S. Effects of prenatal stress and monoaminergic perturbations on the expression of serotonin 5-ht(4) and adrenergic beta(2) receptors in the embryonic mouse telencephalon. Brain Res. 2012, 1459, 27–34. [CrossRef] [PubMed]

107. Schmidt, E.F.; Warner-Schmidt, J.L.; Otopalik, B.G.; Pickett, S.B.; Greengard, P.; Heintz, N. Identification of the cortical neurons that mediate antidepressant responses. Cell 2012, 149, 1152–1163. [CrossRef] [PubMed]

108. Madsen, K.; Torstensen, E.; Holst, K.K.; Haahr, M.E.; Knorr, U.; Frokjaer, V.G.; Iversen, P.; Fisher, P.M.; Knudsen, G.M. Familial risk for major depression is associated with lower striatal 5-ht(4) receptor binding. Int. J. Neuropsychopharmacol. 2014, 18. [CrossRef] [PubMed]

109. Ohitsuuki, T.; Ishiguro, H.; Detera-Wadleigh, S.D.; Toyota, T.; Shimizu, H.; Yamada, K.; Yoshitsugu, K.; Hattori, E.; Yoshikawa, T.; Arinami, T. Association between serotonin 4 receptor gene polymorphisms and bipolar disorder in Japanese case-control samples and the nihm genetics initiative bipolar pedigrees. Mol. Psychiatry 2002, 7, 954–961. [CrossRef] [PubMed]

110. Haahr, M.E.; Fisher, P.M.; Jensen, C.G.; Frokjaer, V.G.; Mahon, B.M.; Madsen, K.; Baare, W.F.; Lehel, S.; Norremolle, A.; Rabiner, E.A.; et al. Central 5-ht4 receptor binding as biomarker of serotonergic tone in humans: A [11c]sb207145 pet study. Mol. Psychiatry 2014, 19, 427–432. [CrossRef] [PubMed]

111. 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. USA 2007, 104, 16335–16340. [CrossRef] [PubMed]

112. Tsang, S.W.; Keene, J.; Hope, T.; Spence, I.; Francis, P.T.; Wong, P.T.; Chen, C.P.; Lai, M.K. A serotoninergic basis for hyperphagic eating changes in Alzheimer’s disease. J. Neurol. Sci. 2010, 288, 151–155. [CrossRef] [PubMed]

113. Bockaert, J.; Marchetti-Gauthier, E.; et al. Chronic treatments with a 5-ht4 receptor agonist decrease amyloid pathology in the entorhinal cortex and learning and memory deficits in the 5xfad mouse model of Alzheimer’s disease. Neuropharmacology 2017, 126, 128–141. [CrossRef] [PubMed]

114. Giannoni, P.; Gaven, F.; de Bundel, D.; Baranger, K.; Marchetti-Gauthier, E.; Roman, F.S.; Valjent, E.; Marin, P.; Bockaert, J.; Rivera, S.; et al. Early administration of rs 67333, a specific 5-ht4 receptor agonist, prevents amyloidogenesis and behavioral deficits in the 5xfad mouse model of Alzheimer’s disease. Front. Aging Neurosci. 2013, 5, 96. [CrossRef] [PubMed]

115. Brodney, M.A.; Johnson, D.E.; Sawant-Basak, A.; Coffman, K.J.; Drummond, E.M.; Hudson, E.L.; Fisher, K.E.; Noguchi, H.; Waizumi, N.; McDowell, L.L.; et al. Identification of multiple 5-ht4 partial agonist clinical candidates for the treatment of Alzheimer’s disease. J. Med. Chem. 2012, 55, 9240–9254. [CrossRef] [PubMed]

116. Madsen, K.; Neumann, W.J.; Holst, K.; Marner, L.; Haahr, M.T.; Lehel, S.; Knudsen, G.M.; Hasselbalch, S.G. Cerebral serotonin 4 receptors and amyloid-beta in early Alzheimer’s disease. J. Alzheimers Dis. 2011, 26, 457–466. [CrossRef] [PubMed]
118. Lai, M.K.; Tsang, S.W.; Francis, P.T.; Esiri, M.M.; Hope, T.; Lai, O.F.; Spence, I.; Chen, C.P. [3h]gr113808 binding to serotonin 5-htr(4) receptors in the postmortem neocortex of alzheimer disease: A clinicopathological study. *J. Neural Transm.* 2003, 110, 779–788. [PubMed]

119. Suzuki, T.; Iwata, N.; Kitamura, Y.; Kitajima, T.; Yamanouchi, Y.; Ikeda, M.; Nishiyama, T.; Kamatani, N.; Ozaki, N. Association of a haplotype in the serotonin 5-htr4 receptor gene (htr4) with japanese schizophrenia. *Am. J. Med. Genet. B Neuropsychiatr. Genet.* 2003, 121B, 7–13. [CrossRef] [PubMed]

120. Li, J.; Wang, Y.; Zhou, R.; Wang, B.; Zhang, H.; Yang, L.; Faraone, S.V. Association of attention-deficit/hyperactivity disorder with serotonin 4 receptor gene polymorphisms in han chinese subjects. *Neurosci. Lett.* 2006, 401, 6–9. [CrossRef] [PubMed]

121. Holmes, A.; Murphy, D.L.; Crawley, J.N. Reduced aggression in mice lacking the serotonin transporter. *Psychopharmacology* 2002, 161, 160–167. [CrossRef] [PubMed]

122. Jennings, K.A.; Loder, M.K.; Sheward, W.J.; Pei, Q.; Deacon, R.M.; Benson, M.A.; Olverman, H.J.; Hastie, N.D.; Harmar, A.J.; Shen, S.; et al. Increased expression of the 5-htr transporter confers a low-anxiety phenotype linked to decreased 5-htr transmission. *J. Neurosci.* 2006, 26, 8955–8964. [CrossRef] [PubMed]

123. Mannoury la Cour, C.; Boni, C.; Hanoun, N.; Lesch, K.P.; Hamon, M.; Lanfumey, L. Functional consequences of 5-htr transporter gene disruption on 5-htr(1a) receptor-mediated regulation of dorsal raphe and hippocampal cell activity. *J. Neurosci.* 2001, 21, 2178–2185. [CrossRef] [PubMed]

124. Gobbi, G.; Murphy, D.L.; Lesch, K.; Blier, P. Modifications of the serotonergic system in mice lacking serotonin transporters: An in vivo electrophysiological study. *J. Pharmacol. Exp. Ther.* 2001, 296, 987–995. [PubMed]

125. Basselin, M.; Fox, M.A.; Chang, L.; Bell, J.M.; Greenstein, D.; Chen, M.; Murphy, D.L.; Rapoport, S.I. Imaging elevated brain arachidonic acid signaling in unanesthetized serotonin transporter (5-htr)-deficient mice. *Neuropsychopharmacology* 2009, 34, 1695–1709. [CrossRef] [PubMed]

126. Qu, Y.; Villacreses, N.; Murphy, D.L.; Rapoport, S.I. 5-htr2a/2c receptor signaling via phospholipase a2 and arachidonic acid is attenuated in mice lacking the serotonin reuptake transporter. *Psychopharmacology* 2005, 180, 12–20. [CrossRef] [PubMed]

127. Jennings, K.A.; Sheward, W.J.; Harmar, A.J.; Sharp, T. Evidence that genetic variation in 5-htr transporter expression is linked to changes in 5-htr2a receptor function. *Neuropsychopharmacology* 2008, 54, 776–783. [CrossRef] [PubMed]

128. Vidal, R.; Valdizan, E.M.; Vilaro, M.T.; Pazos, A.; Castro, E. Reduced signal transduction by 5-htr4 receptors after long-term venlafaxine treatment in rats. *Br. J. Pharmacol.* 2010, 161, 695–706. [CrossRef] [PubMed]

129. Warner-Schmidt, J.L.; Flajolet, M.; Maller, A.; Chen, E.Y.; Qi, H.; Svenningsson, P.; Greengard, P. Role of p11 in cellular and behavioral effects of 5-htr4 receptor stimulation. *J. Neurosci.* 2009, 29, 1937–1946. [CrossRef] [PubMed]

130. Albert, P.R. Why is depression more prevalent in women? *J. Psychiatry Neurosci.* 2015, 40, 219–221. [CrossRef] [PubMed]

131. Perfalk, E.; Cunha-Bang, S.D.; Holst, K.K.; Keller, S.; Svarer, C.; Knudsen, G.M.; Frokjaer, V.G. Testosterone levels in healthy men correlate negatively with serotonin 4 receptor binding. *Psychoneuroendocrinology* 2017, 81, 22–28. [CrossRef] [PubMed]

132. Minett, M.S.; Pereira, V.; Sikandar, S.; Matsuyama, A.; Loliigner, S.; Kanellopoulos, A.H.; Mancini, F.; Iannetti, G.D.; Bogdanov, Y.D.; Santana-Varela, S.; et al. Endogenous opioids contribute to insensitivity to pain in humans and mice lacking sodium channel nav1.7. *Nat. Commun.* 2015, 6, 8967. [CrossRef] [PubMed]

133. Isensee, J.; Krahe, L.; Moeller, K.; Pereira, V.; Sexton, J.E.; Sun, X.; Emery, E.; Wood, J.N.; Hucho, T. Synergistic regulation of serotonin and opioid signaling contributes to pain insensitivity in nav1.7 knockout mice. *Sci. Signal.* 2017, 10. [CrossRef] [PubMed]