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# Role of the 5-HT7 Receptor in the Central Nervous System: from Current Status to Future Perspectives

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**Abstract** Pharmacological and genetic tools targeting the 5-hydroxytryptamine (5-HT)7 receptor in preclinical animal models have implicated this receptor in diverse (patho) physiological processes of the central nervous system (CNS). Some data obtained with 5-HT7 receptor knockout mice, selective antagonists, and, to a lesser extent, agonists, however, are quite contradictory. In this review, we not only discuss in detail the role of the 5-HT7 receptor in the CNS but also propose some hypothetical models, which could explain the observed inconsistencies. These models are based on two novel concepts within the field of G protein-coupled receptors (GPCR), namely biphasic signaling and G protein-independent signaling, which both have been shown to be mediated by GPCR dimerization. This led us to suggest that the 5-HT7 receptor could reside in different dimeric contexts and initiate different signaling pathways, depending on the neuronal circuitry and/or brain region. In conclusion, we highlight GPCR dimerization and G protein-independent signaling as two promising future directions in 5-HT7 receptor research, which ultimately might lead to the development of more efficient dimer- and/or pathway-specific therapeutics.

**Keywords** 5-HT7 receptor · Circadian rhythm · REM sleep · Depression · Thermoregulation · Anxiety · Schizophrenia · Pain · Substance abuse · Memory

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## Introduction

Serotonin (5-hydroxytryptamine, 5-HT) is one of the oldest neurotransmitters in evolution and is implicated in a large variety of behavioral and psychological processes. This is not surprising considering the extensive serotonergic projections in the brain and the large number of serotonin receptor subtypes. Based on pharmacological, structural, and transductional characteristics, the 5-HT receptor family is divided into seven subfamilies (5-HT1–5-HT7), comprising 14 receptor subtypes, each of them corresponding with distinct genes. All the 5-HT receptors are G protein-coupled receptors (GPCRs), except the 5-HT3 receptor, which is a ligand-gated ion channel. The complexity of the 5-HT system is further increased by alternative splicing and mRNA editing of several 5-HT receptors, their potential to form homo- or heterodimers, and crosstalk both within the 5-HT receptor family and with other receptor families [1, 2].

The 5-HT7 receptor is the most recently identified member of the 5-HT receptor family and was cloned independently by three laboratories in 1993 [3–5]. These early studies also showed that the 5-HT7 receptor is positively coupled to adenylate cyclase through the stimulatory $G_s$ protein with a pharmacological profile that

distinguished this receptor from all other 5-HT receptors. This profile is characterized by high affinity for 5-HT and 5-carboxamidotryptamine (5-CT), the 5-HT1A receptor agonist, 8-OH-DPAT, and the 5-HT2 receptor antagonists, ritanserin, metergoline, mesulergine, and risperidone, but not the 5-HT1 receptor antagonist, pindolol [3–6]. Since its discovery, the 5-HT7 receptor has been cloned from rat [3, 4], man [5], mouse [7], guinea pig [8], *Xenopus laevis* [9], *Aedes aegypti* [10], pig [11], *Caenorhabditis elegans* [12], and honeybee [13].

The 5-HT7 receptor promoter contains regulatory elements for AP2, Egr-1, and MAZ and an Sp1/Sp3 consensus motif. In addition, Sp factors were shown to be essential for transcription of the 5-HT7 receptor [14]. The 5-HT7 receptor gene is located on human chromosome 10q21–q24 and contains three introns. Alternative splicing only occurs at the second and third intron and gives rise to at least five splice variants in man, mouse, and rat, which differ in their C-terminal tail. So far, three splice variants have been identified in man (5-HT7a, 5-HT7b, and 5-HT7d) [15], three in mouse (5-HT7a, 5-HT7b, and 5-HT7c) [16], and four in rat (5-HT7a, 5-HT7b, 5-HT7c, and 5-HT7e) [15, 17]. No major differences in their pharmacology nor functionality have been detected so far [16, 18, 19]. RT-PCR studies also rule out large tissue-specific differences in 5-HT7 receptor mRNA splicing, as all the splice variants were found in the same tissues [5, 18, 20]. In general, the 5-HT7a isoform predominates, followed by the 5-HT7b splice variant, while the 5-HT7c and the 5-HT7d isoforms are least frequently expressed [21].

Besides $G\alpha_s$, the 5-HT7 receptor was also shown to interact with $G\alpha_{12}$. Activation of the 5-HT7 receptor/$G\alpha_{12}$ signaling pathway led to stimulation of Cdc42 and RhoA, resulting in serum response element-mediated gene transcription, which in turn led to filopodia formation and cell rounding [22]. The 5-HT7 receptor can also activate extracellular signal-regulated kinase (ERK) [23]; however, the involved signaling pathway seems to differ substantially depending on the cell system. In PC12 cells, 5-HT7 receptor-mediated ERK activation was shown to be dependent on a cAMP-activated guanine nucleotide exchange factor, briefly called Epac, and Rap [24], while in HEK293 cells, the 5-HT7 receptor couples to ERK through protein kinase A (PKA) and Ras [25]. By inducing the rapid phosphorylation of ERK and IκBα in naïve T cells, the 5-HT7 receptor appeared to interact synergistically with T cell receptor signaling in the promotion of T cell activation and proliferation [26]. Finally, stimulation of the 5-HT7 receptor was also shown to induce expression of IL-6 in both an astrocytoma and a microglial cell line [27, 28]. In the astrocytoma cell line, this was shown to be dependent on p38 MAPK and PKCε [28]. Preliminary data indicate that the 5-HT7 receptor can reside in lipid rafts, more specifically caveolae, and internalize in a caveolin-dependent way [29, 30]. The 5-HT7 receptor was also shown to interact with eukaryotic initiation of translation factor 3, subunit k, which seems to play a role in transport of the receptor to the plasma membrane [31]. Very recently, Kvachnina et al. demonstrated that the mouse 5-HT7a receptor is dynamically palmitoylated in an agonist-dependent manner. Mutation analysis revealed that cysteine residues 404 and 438/441 in the C-terminal domain are the main palmitoylation sites and mutation of palmitoylation site Cys404-Ser significantly increased $G_s$-mediated, but not $G_{12}$-mediated constitutive activity of the 5-HT7a receptor [32].

The 5-HT7 receptor is expressed in the central (CNS) and peripheral nervous system (PNS) and also in the periphery. In the latter, the 5-HT7 receptor has been detected predominantly in smooth muscle cells of the cardiovascular [33], gastrointestinal [34], and reproductive system [35, 36], where this receptor was consistently shown to induce smooth muscle relaxation. Both peripheral and centrally localized 5-HT7 receptors were found to play a role in the regulation of the micturition reflex [37–39]. Finally, the 5-HT7 receptor has also been implicated in the neuroendocrine system [40–42]. In the CNS, the 5-HT7 receptor is most abundant in the cortex, thalamus, hypothalamus, and hippocampus [43–46]. The use of 5-HT7 receptor knockout mice, selective antagonists, and, to a lesser extent, selective agonists, in preclinical animal models, has implicated this receptor in a diverse set of CNS functions, including circadian rhythm, rapid eye movement (REM) sleep, depression, thermoregulation, anxiety, schizophrenia, nociception, epilepsy, and memory. For some of these functions, quite contradictory findings have been reported, for which no satisfactory explanation has been given so far. In this review, we discuss in detail the involvement of the 5-HT7 receptor in different (patho)physiological processes of the CNS, which are summarized in Table 1. We also propose some interesting hypothetical models, which could explain the observed discrepancies. These models are based on two relatively new concepts within the GPCR field, namely biphasic signaling and G protein-independent signaling, which both have been shown to be mediated by GPCR dimerization. Hence, we highlight GPCR dimerization as the possible key mechanism that introduces diversity in 5-HT7 receptor signaling. Dimer-based diversification of 5-HT7 receptor signaling could also explain how a single receptor can be involved in such a variety of (patho)physiological processes of the CNS.

**Table 1** Effects of pharmacological and genetic tools targeting the 5-HT7 receptor in preclinical animal models

| (Patho)physiological process | Model | Tool | Effect | References |
|---|---|---|---|---|
| Circadian rhythm: 8-OH-DPAT-induced phase shift | In vitro: neuronal activity of SCN neurons in hyphothalamic slices | SB-269970 | Inhibition | [47] |
| | | KO | Inhibition | [48] |
| | In vivo: wheel running | DR-4004 | Inhibition | [49] |
| | | KO | Inhibition | [50] |
| REM sleep | Electroencephalogram | SB-269970 | Suppression | [51, 52, 105] |
| | | SB-656104 | Suppression | [53] |
| | | LP-44 | Suppression | [54] |
| | | SB-269970+ citalopram | Additional suppressive effect | [55] |
| | | KO | Suppression | [52] |
| Depression | Forced swimming test | SB-258719 | Anti-immobility | [48] |
| | | SB-269970 | Anti-immobility | [52, 56, 57] |
| | | SB-269970, intrahippocampal | Anti-immobility | [58] |
| | | SB-269970+citalopram, imipramine, desipramine or moclobemide[a] | Anti-immobility | [59, 57] |
| | | amisulpride | Anti-immobility | [60] |
| | | KO | Anti-immobility | [48, 52] |
| | Tail suspension test | SB-269970 | Anti-immobility | [52, 56] |
| | | SB-269970+citalopram[a] | Anti-immobility | [55] |
| | | amisulpride | Anti-immobility | [60] |
| | | KO | Anti-immobility | [52] |
| | | KO+citalopram | Additional anti-immobility effect | [52] |
| Thermoregulation | 5-HT-, 5-CT-, or 8-OH-DPAT-induced hypothermia | SB-269970 | Inhibition | [51, 61, 62, 63] |
| | | SB-258719 | Inhibition | [61] |
| | | KO | Inhibition | [61, 62, 64] |
| | Hypoxia-induced hypothermia | SB-269970 | Inhibition | [65] |
| OCD | Marble burying | SB-269970 | Antidepressant-like | [66] |
| | | KO | Antidepressant-like | [66] |
| Anxiety | Elevated plus-maze test | antisense oligonucleotides, KO | No change | [67, 48] |
| | | SB-269970 | Anti-anxiety-like | [56] |
| | Light/dark transfer test | KO | No change | [69] |
| | Conflict drinking test | SB-269970 | Anti-anxiety-like | [56] |
| | Four-plate test | SB-269970 | Anti-anxiety-like | [56] |
| Schizophrenia | Amphetamine (and apomorphine)-disrupted PPT | SB-258741 | No change | [70] |
| | | SB-269970 | Antipsychotic-like | [71] |
| | | KO | No change | [136] |
| | PCP or ketamine-disrupted PPI | SB-258741 | Antipsychotic-like | [70] |
| | | SB-269970 | No change | [71, 136] |
| | | KO | Antipsychotic-like | [136] |
| | PCP-induced reversal learning deficit | SB-269970 | Attenuation | [72] |
| Epilepsy | Absence epilepsy | SB-258719 | Anticonvulsant | [73] |
| | Electrically induced tonic seizures | KO | Proconvulsant | [74] |

**Table 1** (continued)

| (Patho)physiological process | Model | Tool | Effect | References |
|---|---|---|---|---|
| | Chemically induced seizures | KO | Proconvulsant | [74] |
| Pain, peripheral 5-HT7R | Formalin paw-flick test | SB-269970 | Antinociceptive with and without 5-HT/5-CT | [165] |
| Pain, spinal 5-HT7R | Formalin paw-flick test | SB-269970 | Antinociceptive only in presence of low 5-CT doses | [165] |
| | Radiant heat tail-flick test: morphine-induced antinociception | SB-269970 | Pronociceptive | [166] |
| | Thermal paw-flick test: morphine-induced antinociception | SB-269970 | Pronociceptive | [167] |
| | Radiant heat tail-flick test: tramadol-induced antinociception | SB-269970, SB-258719 | Pronociceptive | [75] |
| | Plantar incision test: tramadol-induced antinociception | SB-269970, SB-258719 | Pronociceptive | [75] |
| Pain, supraspinal 5-HT7R | Electrical tail-flick test: 8-OH-DPAT-induced antinociception | SB-269970 | Pronociceptive (only on vocalizations) | [76] |
| | Capsaicin-induced mechanical hypersensitivity | E-55888, AS-19 and MSD-5a | Antinociceptive | [77] |
| | | SB-269970, SB-258719 | Pronociceptive, with and without E-55888, AS-19 and MSD-5a | [77] |
| | Radiant heat tail-flick test | KO | No change | [69] |
| Migraine | 5-CT-induced dilatation of the middle meningeal artery | SB-269970 | Inhibition | [78, 79] |
| Sensation-Seeking and Impulsive Behavior | NOD test | SB-269970 | Reduction of novel object exploration time | [80] |
| | Impulsitivity | SB-269970 | Counteracts MPH-reduced impulsivity | [81] |
| | | | Enhances impulsivity | [81] |
| Memory | Radial arm maze test (working memory and STM) | SB-269970 | No effect on working memory<br>Improvement STM | [82] |
| | Associative learning task (STM, LTM, and memory consolidation) | AS-19 | Attenuation STM<br>Improvement LTM and memory consolidation | [83] |
| | | SB-269970 | Alone: no effect<br>Inhibition AS-19-facilitatory and inhibitory effect on LTM and STM, respectively | |
| | Pavlovian/instrumental autoshaping learning task (LTM) | SB-269970, DR-4004 | Inhibition 8-OH-DPAT-facilitatory effect | [84, 85] |
| | | DR-4004 | Alone: no effect | [84] |
| | | AS-19 | Improvement | [86] |
| | | SB-269970 | Inhibition AS-19-facilitatory effect | |
| | Scopolamine- and dizocilpine-induced amnesia | SB-269970, DR-4004 | Inhibition | [85] |
| | Motor learning | KO | No effect | [69] |
| | Cued conditioning | KO | No effect | |

**Table 1** (continued)

| (Patho)physiological process | Model | Tool | Effect | References |
|---|---|---|---|---|
| | Operant conditioning | KO | No effect | |
| | Barnes maze test: spatial learning | KO | No effect | |
| | Contextual fear conditioning | KO | Impairment | |
| | Passive avoidance task: aversive contextual learning | SB-269970 | Intensification of 8-OH-DPAT induced impairments<br>Alone: no effect | [87] |
| | Novel object test | KO<br>SB-269970 | No effect<br>No effect | [125] |
| | Novel location test | KO | Reduced sensitivity for spatial changes | |
| | | SB-269970 | Reduced sensitivity for spatial changes | |
| | Modified Barnes maze test:<br>Acquisition phase<br>Probe test<br>Retention test<br>Reversal test | KO | Acquisition phase and retention test: no effect<br>Probe test and reversal test: impairment allocentric memory | |

[a] Ineffective doses

## Circadian Rhythm

In mammals, circadian rhythms, such as sleep–wake cycle, body temperature changes, hormonal releases, and cyclic behavioral patterns, are regulated by "the master biologic clock", which is located in the suprachiasmatic nucleus (SCN). The activity of the SCN is synchronized to the light/dark cycle by means of photic information, which reaches the SCN through the retinohypothalamic tract. In addition, neuropeptide Y- and serotonin-containing projections have been shown to modulate SCN activity non-photically. The serotonergic innervation of the SCN follows a multisynaptic dorsal raphe nucleus (DRN)–median raphe nucleus (MRN)–SCN route [88].

### Non-photic Phase Shifting

Immediately after its discovery, the 5-HT7 receptor was shown to mediate the 8-OH-DPAT-induced non-photic phase resetting of the neuronal activity of SCN neurons in vitro, which was suppressed by the 5-HT7/2 antagonist ritanserin, but not by the 5-HT1A/1B antagonist, pindolol [3]. The involvement of the 5-HT7 receptor was further confirmed in a study of Sprouse et al., in which SB-269970, a more selective 5-HT7 receptor antagonist, effectively antagonized the 8-OH-DPAT-induced phase advance [47]. Ehlen et al. showed as first that a circadian phase shift in response to 8-OH-DPAT in vivo was also dependent on 5-HT7 receptor activation by means of ritanserin and the more selective 5-HT7 receptor inhibitor, DR-4004 [49]. According to Horikawa et al., this phase shift is associated with a reduction in Per1 and Per2 mRNA in the SCN [89]. 8-OH-DPAT also induces a phase shift in the circadian rhythm in mice, but in contrast to hamsters, only in a very narrow time span, namely exclusively in the middle of the subjective day at circadian time 6 [90], and for this reason was missed in the study of Antle et al. [91]. Guscott et al. then compared the responses of hypothalamic slices to 8-OH-DPAT from WT and 5-HT7 receptor KO mice and observed significant phase shifts in slices from WT mice, but not in those from 5-HT7 receptor KO mice, which indicates dependence on the 5-HT7 receptor [48]. This was confirmed in vivo by the study of Gardani and Biello, who observed a significant phase shift by 8-OH-DPAT in WT mice, which was strongly attenuated in 5-HT7 receptor KO mice [50].

Finally, the 5-HT7 receptor in the DRN has also been shown to play an important role in aging-related deficits of the circadian time-keeping system. The expression level of the 5-HT7 receptor in the DRN specifically decreases with

aging in hamsters [92], which has later been shown to cause a reduced sensitivity to 8-OH-DPAT with regard to phase resetting in elderly hamsters [93].

### Suppression of Photic Activation of the SCN

5-HT not only causes non-photic phase shifts of SCN activity but also influences the response of SCN neurons to light [94]. Ying and Rusak found that 5-HT, 5-CT, and 8-OH-DPAT dose-dependently reduced photic activation of hamster SCN cells, which was blocked by co-application of ritanserin and clozapine. Cyanopindolol, a 5-HT1A/B/D antagonist, and WAY-100635, a 5-HT1A antagonist, on the other hand had no antagonist effects on the response to 8-OH-DPAT [95]. Additionally, Smith et al. studied the effect of a mixed 5-HT1A/B agonist, "TFMPP," on the amplitude of glutamatergic excitatory postsynaptic currents (EPSCs), evoked by stimulation of the optic nerve in mice hypothalamic slices in vitro. The agonist reduced the amplitude of the optic nerve-evoked EPSCs in slices derived from WT mice but had a negligible effect in slices from a 5-HT1B receptor KO mouse, stressing the importance of the 5-HT1B receptor in this process. 8-OH-DPAT was also found to suppress the EPSCs following optic nerve stimulation, both in WT and 5-HT1B-KO mice. This effect was minimally attenuated by WAY-100635 but effectively blocked by ritanserin, indicating dependence on 5-HT7 receptor activation [96]. Thus, all together, these data suggest that the inhibitory effect of 5-HT on the activation of SCN neurons in response to light can at least in part be mediated by the 5-HT7 receptor. However, it should be noted here that the putative 5-HT7 receptor agonist, AS-19, failed to inhibit light-induced phase shifts after systemic administration [97]. It is possible that the dose used in this study (5 mg/kg) was too low to affect the SCN clock, even though the same dose was able to impair short-term memory, improve both long-term memory and memory consolidation [83, 98], and inhibit capsaicin-induced mechanical hypersensitivity [77]. There is also some uncertainty concerning the selectivity of AS-19 [99].

### Regulation of 5-HT Release in the SCN

So, it is clear that 5-HT is able to modulate SCN activity and that the 5-HT7 receptor probably plays an important role in this, but how is 5-HT release in the SCN regulated or, in other words, what is the physiological role of 5-HT-mediated modulation of SCN activity? In this regard, Glass et al. showed that 5-HT release in the SCN is induced by electrical stimulation of the DRN and that this is attenuated by intra-DRN or intra-MRN injection of DR-4004 and the $GABA_A$ agonist muscimol and stimulated by the $GABA_A$ antagonist bicuculline. Secondly, they demonstrated that novel wheel access also induces phase-advance shifts in the SCN and that these are likewise suppressed by intra-DRN injection of DR-4004 and muscimol [88]. All together, these data suggest that certain behavioral stimuli can trigger electrical activity of the DRN, leading to 5-HT release and subsequent activation of 5-HT7 receptors in both the DRN and MRN, which ultimately results in 5-HT release in the SCN and phase shifting of SCN activity. GABAergic neurons in both the DRN and MRN seem to play an inhibitory role in this. So, a possible physiological role for 5-HT release in the SCN could be the mediation of phase shifts of SCN activity in response to behavioral changes, which is in agreement with earlier observations that behavioral phase-resetting manipulations evoke 5-HT release in the SCN [100, 101].

It has been shown that GABA-immunoreactive terminals synapse with serotonin-positive neurons in the DRN, which express $GABA_A$ receptors [102, 103]. By means of muscimol and bicuculline, Roberts et al. showed that the GABAergic neurons inhibit neuronal serotonergic activity and 5-HT release in the DRN. Moreover, they demonstrated that SB-269970 also suppresses electrically stimulated 5-HT release in the DRN, which was attenuated in the presence of bicuculline [68]. This suggests that 5-HT7 receptor activation leads to inhibition of GABA interneurons, thereby decreasing GABA release and hence reducing the inhibitory tone on 5-HT neurons. This is in agreement with the data of Duncan et al., which indicate that 5-HT7 receptors in the DRN are not located on serotonergic neurons but instead may reside on GABA interneurons [104]. All together, these data suggest a mutual inhibition between serotonergic and GABAergic neurons in the DRN and a central role for 5-HT7 receptors in the inhibition of the GABAergic neurons. Considering the similar inhibitory effect of DR-4004 and muscimol and stimulatory effect of bicuculline in the DRN on 5-HT release in the SCN, the latter is most probably also regulated by mutually inhibiting serotonergic and GABAergic neurons in the DRN. To summarize, the following model is proposed: Electrical stimulation of serotonergic neurons in the DRN leads to initial 5-HT release and subsequent 5-HT7 receptor-mediated inhibition of GABAergic neurons, relieving GABA-mediated inhibition of the serotonergic neurons, which leads to a further increase of serotonergic activity and 5-HT release. The increasing activation of serotonergic neurons in the DRN ultimately reaches the serotonergic terminals in the

SCN, where 5-HT is released and modulates the SCN neurons. Interestingly, it is well-known that the sleep/wake cycle is regulated by a flip/flop system of mutually inhibiting monoaminergic and GABAergic neurons, promoting wakefulness and sleep, respectively. So, the serotonergic and GABAergic neurons in the DRN could belong to this system and use the DRN–MRN–SCN route to send feedback information concerning the sleep/wake state of the organism to the SCN. In case of a desynchronization between SCN activity and sleep/wake state, 5-HT or a lack of 5-HT possibly modulates SCN activity, for example, by causing a reset in the circadian phase (see Fig. 1).

## REM Sleep: Biphasic Regulation by the 5-HT7 Receptor?

The 5-HT7 receptor also seems to play an important role in the regulation of REM sleep. Hagan et al. and Thomas et al. showed that systemic administration of respectively SB-269970 and SB-656104, two 5-HT7 receptor antagonists, to rats at the beginning of the light (sleep) period reduced the total amount of REM sleep, whereas wakefulness (W) and slow wave sleep (SWS) were not significantly modified [51, 53]. In agreement with this, 5-HT7 receptor knockout mice spent less time in REM sleep compared with 5-HT7$^{+/+}$ mice, while no difference was seen in time spent in W or SWS [52]. In addition, Monti and Jantos demonstrated that micro-injection of SB-269970 into the DRN of rats suppressed REM sleep, which was prevented by pretreatment with muscimol [105]. This suggests that activation of the 5-HT7 receptor in the DRN promotes REM sleep by stimulation of GABA release and its subsequent binding on the $GABA_A$ receptor. Although this is in line with GABAergic neurons generally being considered as REM stimulatory, it contradicts the observed mutual inhibition between serotonergic and GABAergic neurons in the DRN by Roberts et al., in which the 5-HT7 receptor was shown to inhibit GABA release [68]. In agreement with this, serotonergic neurons from the DRN have been shown to be most active during wake state, slow down their firing during SWS and completely cease firing specifically during REM sleep. Moreover, the three major classes of antidepressant drugs, monoamine oxidase inhibitors (MAOIs), tricyclic antidepressants (TCAs), and selective serotonin reuptake inhibitors (SSRIs), all enhance serotonergic transmission and profoundly suppress REM sleep. MAOIs virtually completely abolish REM sleep, while TCAs and SSRIs have been shown to produce immediate (40–85%) and sustained (30–50%) reductions in REM sleep. So, in order to check the influence of specific 5-HT7 receptor activation on REM

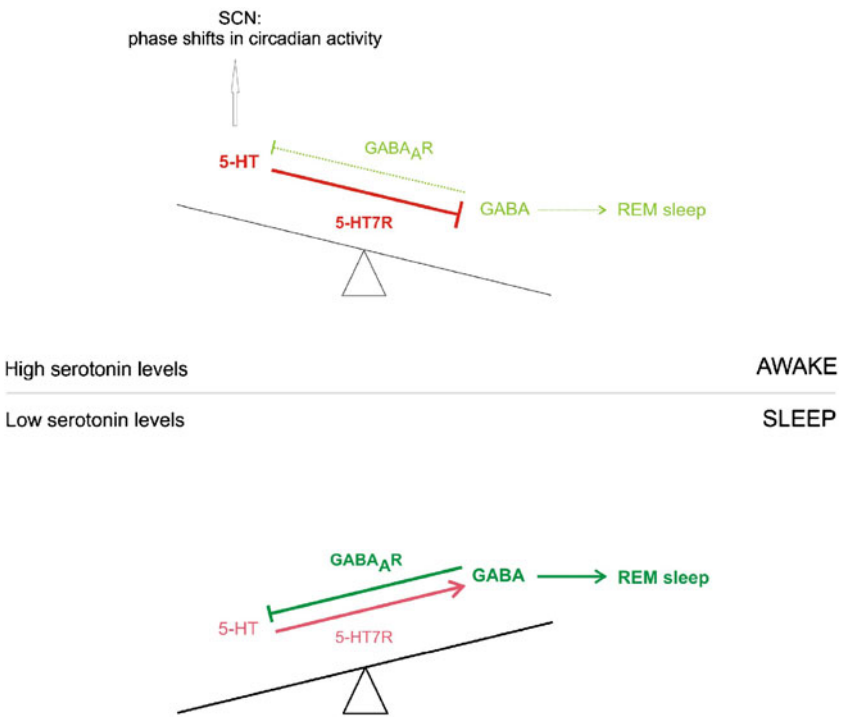


**Fig. 1** Hypothetical model of 5-HT7 receptor-mediated biphasic regulation of REM sleep and 5-HT release. The 5-HT7 receptor in the DRN regulates GABAergic neurons in a biphasic manner, which in turn inhibit serotonergic activity and 5-HT release and stimulate REM sleep. The serotonergic and GABAergic neurons in the DRN most probably belong to the flip/flop regulation system of the sleep/wake cycle, promoting wakefulness and sleep, respectively. In case of a desynchronization between sleep/wake state and SCN activity, 5-HT or a lack of 5-HT in the DRN could cause a phase shift in SCN activity

sleep, Monti et al. micro-injected LP-44, a selective 5-HT7 agonist, into the DRN and noticed that, like SB-269970, LP-44 also suppresses REM sleep [54]. A possible explanation for these contradictory findings could be that GABAergic "REM-on" neurons are indeed stimulated by 5-HT7 receptors, but only in the absence of 5-HT and/or at low 5-HT concentrations, while at high 5-HT concentrations, 5-HT7 receptors inhibit these GABAergic neurons (see Fig. 1). So, a concentration-dependent switch in 5-HT7 receptor signaling, also generally referred to as "biphasic activity," could explain why both inhibition of 5-HT7 receptor activity, as well as elevated 5-HT concentrations or administration of a highly concentrated 5-HT7 receptor agonist, prevent 5-HT7 receptor-mediated stimulation of the REM stimulatory GABAergic neurons. Similarly, both agonists and antagonists of the 5-HT1A receptor, as well as of the 5-HT2A/2C receptor, have been reported to have inhibitory effects on REM sleep [106, 107]. Interestingly, Monti et al. earlier also observed biphasic activity of dopamine (DA) D2 receptor agonists with regard to regulation of sleep and wakefulness, with low doses decreasing W and increasing SWS and REM sleep and high doses inducing the opposite effects [108].

Box 1: Possible biphasic regulation of 5-HT release by the 5-HT7 receptor.

If 5-HT7 receptors in the DRN indeed modulate GABAergic activity in a biphasic way, then 5-HT7 receptor activation would also have an opposite effect on 5-HT release depending on 5-HT concentration, with high and low levels of 5-HT respectively further increasing and decreasing 5-HT release (see Fig.1). As previously mentioned, SB-269970 was shown to have a significant inhibitory effect on 5-HTrelease in both rat and guinea pig DRN slices after electrical stimulation at 20 Hz [68, 109]. However, in a previous study, in which 5-HT efflux was evoked by electrical stimulation at 100 Hz, the same authors could not demonstrate an effect of SB-269970 in the rat DRN. The 20-Hz stimulation burst has a 1-s duration, five times that of the 100-Hz burst, and is thought to generate more endogenous 5-HT tone in the slice [110]. So, only under conditions of high endogenous 5-HT tone the 5-HT7 receptor was shown to have a positive influence on serotonergic activity, most probably through inhibition of GABA-release [68]. In line with this, systemic administration of AS-19 was shown to have a biphasic effect on 5-HT release in the ventral hippocampus of rat, decreasing 5-HT release at low doses, while further increasing 5-HT release at high doses [99]. Because the selectivity of AS-19 for the 5-HT7 receptor compared with the 5-HT1A receptor appeared to be only moderate, the 5-HT1A antagonist, WAY-100635 was also added in this experiment. Similar effects were observed after micro-injection or -dialysis of 8-OH-DPAT into the MRN, which induced a U-shaped dose-effect on extracellular 5-HT both locally and in the hippocampus [111].

Box 2: Biphasic activity mediated by 5-HT7 receptor dimerization?

Very recently, Liu *et al.*observed an agonist concentration-dependent switch in coupling of the β2-adrenergic receptor from $G_s$ to $G_i$. Only at high concentrations of isoproterenol the β2-adrenergic receptor is phosphorylated by GRK, which is necessary for binding to $G_i$(112). The mechanism through which only high concentrations of agonist induce GRK-dependent phosphorylation of this receptor, leading to a switch from $G_s$-to $G_i$-coupling, is currently not known. Structural studies reveal that higher concentrations of agonist induce distinct conformational changes than those induced by low concentrations (113), which may enhance the binding affinity of the receptor for GRK. In this study biphasic activity was proposed to result from the different affinities of isoproterenol for $G_s$-pre-coupledand non-coupled β2-adrenergic receptors. Low concentrations may selectively activate the $G_s$-pre-coupled pool of receptors through high affinity binding, whereas higher concentrations of agonists activate both the pre-coupled and non-coupled pools of receptors. The latter pool of receptors may be involved in coupling to $G_i$proteins. Alternatively, a concentration-dependent switch in receptor activity may be caused by an antagonistic homodimerization. Low concentrations of isoproterenol would then activate only one dimer protomer, leading to activation of $G_s$, while higher concentrations of isoproterenol would also activate the second dimer partner, which leads to GRK phosphorylation followed by $G_i$-coupling and trans-inhibition of the $G_s$-coupled protomer. Yet another possible mechanism for a concentration-dependent switch in GPCR signaling could be an antagonistic heterodimerization between a $G_s$-coupled receptor and a $G_i$-coupled receptor with different affinities for the same agonist, as has been shown for adenosine $A_1$and $A_{2A}$receptors (114). In case of the 5-HT7 receptor, low levels of 5-HT would then only activate the $G_s$-coupled 5-HT7 receptor, leading to stimulation of GABA release, while high concentrations of 5-HT would then also bind to a $G_i$-coupled 5-HT receptor, which not only inhibits GABAergic neuronal activity, but also 5-HT7 receptor activity. Interestingly, preliminary results from our labindicate that the 5-HT7receptor is able to form heterodimers with the $G_i$-coupled 5-HT1A receptor (unpublished data).

## Depression

As 5-HT7 receptor-selective antagonists appeared to alter REM sleep parameters in the same way as certain antidepressants and in a pattern opposite from that seen in patients with clinical depression, the role of the 5-HT7 receptor in the latter was further investigated. 5-HT7 receptor knockout mice not only showed a reduced REM sleep but also exhibited antidepressant-like behavior in two frequently used animal models of depression, namely the forced swim test (FST) and tail suspension test (TST) [48, 52]. In agreement with this, systemic administration of SB-

269970 to mice evoked an anti-immobility effect in both the FST and TST [52, 56]. Very recently, 5-HT7 receptor antagonism has been shown to be clinically relevant for the treatment of depression. The antidepressant effect of the atypical antipsychotic, amisulpride, had traditionally been ascribed to its antagonism at the D2/D3 dopamine receptor, but this mechanism had never been satisfactorily explained. Very recently, amisulpride was shown to be a potent antagonist at the human 5-HT7a receptor and, moreover, appeared to reduce immobility in both the TST and FST in 5-HT7$^{+/+}$ mice, but not in 5-HT7 receptor$^{-/-}$ mice [60]. This indicates that 5-HT7a receptor antagonism, and not D2/D3 antagonism, likely underlies the antidepressant actions of amisulpride. Interestingly, aripiprazole, another atypical antipsychotic, also binds to the 5-HT7 receptor with high affinity and has been successfully used to augment the effect of traditional antidepressants [115, 116]. Moreover, a pharmacological diverse range of antidepressants, namely mianserin, maprotiline, imipramine, and amitriptyline, but not fluoxetine, appeared to behave as antagonists at enteric 5-HT7 receptors either through competitive or allosteric mechanisms [117].

One of the brain structures that has received much attention with regard to a possible role in both the etiology and treatment of depression is the hippocampus. Stress and elevated glucocorticoid levels, which both can produce depression, appeared to be potent inhibitors of hippocampal cell proliferation [118, 119]. Moreover, most antidepressants and environmental interventions that confer antidepressant-like behavioral effects have been shown to stimulate precursor cell proliferation in the dentate gyrus of the hippocampus [120–123]. Santarelli et al. demonstrated that hippocampal cell proliferation was actually necessary for the antidepressant-like effects of imipramine and fluoxetine in two mouse behavioral screens for antidepressant activity [124]. Interestingly, intrahippocampal administration of SB-269970 in rats had an anti-immobility effect in forced swimming tests comparable to that of imipramine [58], which suggests that like imipramine, SB-269970 also exerts its antidepressant-like effect through stimulation of hippocampal cell proliferation. However, this seems very unlikely, as no difference could be observed in the number of proliferating cells in the subgranular zone of the dentate gyrus between 5-HT7$^{+/+}$ and 5-HT7$^{-/-}$ mice [125]. In this regard, Kvachnina et al. showed that treatment of hippocampal neurons with 5-CT led to pronounced extension of neurite length, which was blocked by SB-269970 [22]. So, the 5-HT7 receptor could be involved in cell differentiation and/or synaptic plasticity of newborn adult neurons in the hippocampus rather than cell proliferation.

It should be noted that in mice [56] as well as in rats [57, 58], SB-269970 induces anti-immobility following a U-shaped dose–response relationship. The lack of typical dose dependence or, more specifically, the reduced effectiveness of higher doses of SB-269970 could be due to non-specific activity of SB-269970. However, so far, there are no data showing such non-specific action of SB-269970 [51, 126].

Mood improvement, induced by several serotonin-raising antidepressants (fluoxetine, tranylcypromine, and imipramine), has been shown to be dependent on 5-HT availability [127], and so, the therapeutic efficacy of these classical antidepressants could be mediated by an increased activation of the 5-HT7 receptor, amongst others. If so, then again both inhibition and stimulation of 5-HT7 receptor activity lead to the same antidepressant(-like) phenotype, as has previously been shown to be the case for REM sleep regulation. Likewise, this could be explained by biphasic activity of the 5-HT7 receptor, stimulating depressive(-like) behavior only at low 5-HT levels. Hence, switching 5-HT7 receptor activity by raising 5-HT levels as well as antagonist-mediated inhibition of 5-HT7 receptor activity would bypass the depression-inducing activity of the 5-HT7 receptor.

## Combined Treatment with Classical Antidepressants and SB-269970

Although SSRIs are clinically effective antidepressants, most patients do not show signs of mood improvement until 2 to 3 weeks after the start of the treatment [128]. Moreover, about one third of these patients show only partial or no response to the treatment. Side effects are also commonly reported during chronic treatment, notably insomnia, somnolence, dizziness, akathisia, and long-term sexual dysfunction [129]. In order to improve the activity of the conventional antidepressant drugs, several strategies are in progress, including additional blockade of aminergic autoreceptors, such as the 5-HT1A and/or 5-HT1B receptor, or antagonism at certain postsynaptic receptors, such as 5-HT2A/2C receptors or 5-HT7 receptors [55]. Combined treatment of mice with ineffective doses of citalopram and SB-269970 resulted in a significant decrease of immobility time in both the FST and TST [55, 59]. SB-269970 was also shown to potentiate the delayed onset and decrease in REM sleep induced by citalopram in rats [55]. As citalopram and SB-269970 did not affect each other's concentrations in the plasma and brain, Bonaventure et al. ruled out a possible pharmacokinetic interaction between SB-269970 and citalopram in favor of a pharmacodynamic interaction. Ineffective doses of both SB-269970 and several other classes of antidepressants, namely the tricyclic 5-HT/noradrenaline (NA) reuptake inhibitor, imipramine; the selective noradrenaline reuptake inhibitor, desipramine; and the monoamine oxidase-A inhibitor, moclobemide, also significantly reduced immobility in the FST in mice [59]. In addition, a statistically significant anti-immobility effect was also observed in rats in the FST after administration of

ineffective doses of SB-269970 and imipramine [57]. It should be noted that none of the compounds used, neither alone or in combination, increased the spontaneous locomotor activity of rats, as evaluated by the open field test [57, 59]. Finally, citalopram has been shown to decrease immobility in the TST not only in wild-type mice but also in 5-HT7 receptor knockout mice, in which the effect appeared to be additive to that of the genotype alone [52].

### The Effect of SB-269970 or SB-258741, Administered Alone or with Classical Antidepressants, on 5-HT Release

As mentioned before, mood improvement in depressed patients that positively respond to treatment with various classes of serotonin-raising antidepressant drugs was rapidly impaired by inhibition of 5-HT synthesis [127]. So, at least for some antidepressants, 5-HT is crucial for their therapeutic efficacy. In this regard, several studies have examined the effect of SB-269970, administered alone or with classical antidepressants, on 5-HT release. In the study by Wesolowska and Kowalska, SB-269970 was not only shown to induce an anti-mobility effect but also increased the efflux of DA, NA, and 5-HT in the prefrontal cortex of rats [57]. The SB-269970-induced increase in 5-HT release could be mediated by inhibition of 5-HT7 receptors localized on GABAergic neurons. How DA and NA release is regulated by the 5-HT7 receptor is at present unclear. Inhibition of 5-HT7 heteroreceptors on DA and NA neurons is a possibility, but there is no evidence in literature pointing to such localization of 5-HT7 receptors. In the same study, it was also shown that combined administration of SB-269970 and imipramine at doses ineffective in the FST enhanced NA release but did not influence 5-HT efflux and even decreased DA levels, when compared with the levels observed after treatment with these compounds separately. It was concluded that an increased cortical NA level could in part account for the behavioral enhancement observed after combined administration of SB-269970 and imipramine to rats. In line with these data, Bosker et al. showed that the selective 5-HT7 receptor antagonist, SB-258741, did not modify 5-HT release in the ventral hippocampus of the rat in response to citalopram alone. However, SB-258741 significantly reduced 5-HT release in the ventral hippocampus as well as 5-HT neuronal firing in the DRN, induced by combined administration of citalopram and WAY-100635 [99]. So, it seems that a 5-HT7 receptor antagonist on itself decreases 5-HT release, but when co-administered with a serotonin-raising antidepressant, it does not influence 5-HT release anymore. In addition, when co-administered with both citalopram and the 5-HT1A antagonist, WAY100636, which further raises the extracellular 5-HT concentration, a significant inhibitory effect was observed. These differential effects of 5-HT7 receptor antagonism on 5-HT release is in fact consistent with the previously described biphasic model, in which 5-HT7 receptors exert opposing effects on GABAergic neurons and 5-HT release depending on the extracellular 5-HT concentration (see also boxes 1 and 2).

## Thermoregulation

The first evidence supporting a role for the 5-HT7 receptor in thermoregulation was delivered in a characterization study of the 5-HT7 receptor-selective antagonist, SB-269970 [51]. In that report, it was demonstrated that 5-CT (0.3 mg/kg)-induced hypothermia in guinea pigs was blocked by both SB-269970 and the non-selective 5-HT7 receptor antagonist, metergoline, but not by the 5-HT1A/1B antagonist, pindolol, nor the 5-HT1B/1D antagonist, GR-125743 [51]. Two subsequent reports, which used two different 5-HT7 receptor knockout mouse strains, confirmed the hypothesis that 5-HT-induced hypothermia is mediated by the 5-HT7 receptor [61, 64]. The first study demonstrated that 5-CT (0.1, 0.3, and 1 mg/kg) failed to induce hypothermia in 5-HT7$^{-/-}$ mice and that 5-CT (0.3 mg/kg)-induced hypothermia in 5-HT7$^{+/+}$ mice was reversed by the 5-HT7 receptor antagonists, SB-269970 and SB-258719, but not by the 5-HT1A receptor antagonist, WAY-100635, nor the 5-HT1B/1D receptor antagonist, GR-127935 [61]. The second study showed that both 5-HT (5 mg/kg) and 5-CT (0.5 mg/kg) were unable to induce hypothermia in 5-HT7$^{-/-}$ mice [64]. However, a significant decrease in temperature could still be observed in the 5-HT7$^{-/-}$ mice after treatment with a higher dose of 5-CT (3 mg/kg), although this decrease was significantly less pronounced than in the 5-HT7$^{+/+}$ mice. Nevertheless, this suggests the involvement of another 5-HT receptor at higher doses of 5-CT. Interestingly, several earlier studies pointed out a role for the 5-HT1A receptor in thermoregulation, such as the study of Heisler et al., in which 8-OH-DPAT (0.2 and 1 mg/kg) was not able to induce hypothermia in 5-HT1A$^{-/-}$ mice [130]. In order to determine the relative contribution of the 5-HT1A and 5-HT7 receptor in 8-OH-DPAT-induced hypothermia, Hedlund et al. studied the effects of 8-OH-DPAT, with or without selective antagonists for these receptors, on body temperature in rats and mice, including 5-HT7$^{-/-}$ mice. At lower doses (0.3–0.6 mg/kg, i.p.), 8-OH-DPAT decreased body temperature in 5-HT7$^{+/+}$ mice, but not in 5-HT7$^{-/-}$ mice. SB-269970 fully inhibited and inhibited for 60% hypothermia induced by 0.3 mg/kg 8-OH-DPAT in 5-HT7$^{+/+}$ mice and rats, respectively. At a higher dose (1 mg/kg, i.p.), 8-OH-DPAT induced hypothermia in both 5-HT7$^{-/-}$ and 5-HT7$^{+/+}$ mice. WAY-100635 (10 mg/kg), but not SB-269970 nor DR-4004, fully inhibited the effect of this higher dose of 8-OH-DPAT in both mice and rats [62]. In conclusion, in rodents, 8-OH-DPAT-induced hypothermia is

mediated by both the 5-HT7 and 5-HT1A receptor at, respectively, low and high doses of 8-OH-DPAT. This is in agreement with the previous observation of Hedlund et al., which showed that 5-CT completely failed to induce hypothermia in 5-HT7$^{-/-}$ mice at a lower dose, while a higher dose of 5-CT still induced a modest, but significant decrease in body temperature [64]. All together, these data suggest that hypothermia is induced by both the 5-HT7 and 5-HT1A receptor, each stimulated by different 5-HT concentrations. If so, the 5-HT7 receptor would then respond to rather small 5-HT increases in order to maintain temperature homeostasis, while the 5-HT1A receptor would only get activated in presence of sufficiently high levels of 5-HT to protect the organism from hyperthermia. A possible molecular mechanism for this dose-dependent activation of these two 5-HT receptors could be an antagonistic heterodimerization between the 5-HT7 receptor and 5-HT1A receptor, displaying different affinities for 5-HT (Fig. 2). Briefly, low 5-HT concentrations would only activate the 5-HT7 receptor, while higher levels of 5-HT would then stimulate the 5-HT1A receptor, which could inhibit 5-HT7 receptor activity allosterically. As mentioned previously, preliminary data from our lab indicate that the 5-HT7 receptor and 5-HT1A receptor can indeed form heterodimers (unpublished data). Remarkably, Hedlund et al. found that WAY-100635 also fully inhibited 8-OH-DPAT-induced hypothermia at the lower doses, which was actually shown to be dependent on 5-HT7 receptor activity [62]. Considering the proposed hypothetical model of an antagonistic heterodimerization between the 5-HT1A and 5-HT7 receptor, WAY-100635 could block 5-HT7 receptor activity through binding to the 5-HT1A receptor. Reversely, however, SB-269970 did not seem to inhibit 8-OH-DPAT-induced hypothermia at a higher dose, which was shown to be mediated by the 5-HT1A receptor. Similarly, knockout of the 5-HT1A receptor abolished 8-OH-DPAT-induced hypothermia also at a lower dose [130], while 5-HT7 receptor knockout did not affect hypothermia in response to a higher dose of 8-OH-DPAT [62]. It should be noted here that 5-CT (0.3 mg/kg)-induced hypothermia was not inhibited by pindolol in guinea pigs [51] nor by WAY-100635 (0.1–1 mg/kg) in mice [61]. In contrast to Guscott et al., however, Hedlund et al. used higher concentrations of WAY-100635 (10 mg/kg) [62]. In addition, Faure et al. not only showed that SB-269970 dose-dependently prevented 8-OH-DPAT (0.1 mg/kg)-induced hypothermia in rats but also that co-injection of WAY-100635 together with SB-269970 reduced 8-OH-DPAT-induced hypothermia in an additive manner [63].

Finally, micro-injection of WAY-100635 as well as SB-269970 in the rat anteroventral preoptic region (AVPO) was shown to attenuate hypothermia in response to hypoxia, which indicates that both the 5-HT7 and 5-HT1A receptors in the AVPO are involved in hypoxia-induced hypothermia [65].

## Obsessive–Compulsive Disorder

Obsessive–compulsive disorder (OCD) is a mental disorder characterized by intrusive thoughts that produce anxiety (obsessions) and repetitive behaviors aimed at reducing anxiety (compulsions). This disorder has been proven very difficult to model in laboratory animals, which is demonstrated by the large number of models tried out [66]. Marble burying is one of the more well-established models for OCD; however, as anxiolytic agents, such as diazepam and buspirone, have been shown to reduce burying behavior, some investigators consider this more as a model for anxiety [131, 132]. The current pharmacological treatment of choice for OCD is antidepressants, specifically SSRIs [133]. As blockade or inactivation of the 5-HT7 receptor also produces antidepressant-like effects, similar manipulations could be of interest to study in models of OCD. In this regard, Hedlund and Sutcliffe found that, like antidepressants, both inactivation and blockade of the 5-HT7 receptor in mice reduce the stereotypical behavior of marble

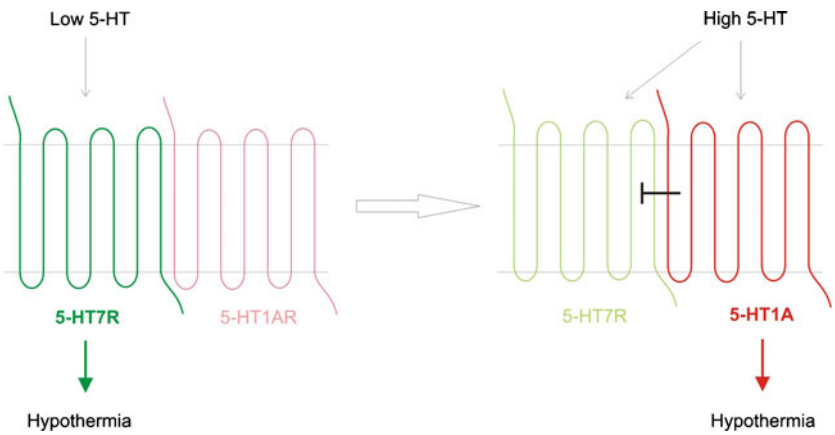


**Fig. 2** Hypothetical model of the induction of hypothermia by an antagonistic 5-HT7R/5-HT1AR-heterodimer. 5-HT-induced hypothermia is mediated by the 5-HT7 receptor at low 5-HT levels, while higher levels of 5-HT also activate the 5-HT1A receptor, which also induces hypothermia and allosterically inhibits 5-HT7 receptor activity

burying [66]. Thus, antagonism at the 5-HT7 receptor might also be a valuable approach for the treatment of OCD.

## Anxiety

So far, only a few papers are available on the effect of 5-HT7 receptor manipulation in animal models of anxiety. An early study by Clemett et al. showed that treatment of rats with 5-HT7 antisense oligonucleotides had no significant effect on the percentage of open entries or time spent in the open arms in the elevated plus-maze test [67]. In the same model, which is a procedure based on rodents' natural aversion to heights and open spaces, both wild-type and 5-HT7 receptor knockout mice showed no difference in the time spent exploring the open arms or in the number of entries into the open arms of the maze [48]. Similarly, innate anxiety-like behavior, as determined by the light/dark transfer test, was not altered in 5-HT7 receptor knockout mice [69]. On the contrary, Wesolowska et al. showed that systemic administration of SB-269970 produced an anxiolytic-like effect in two conflict procedures (the conflict drinking test in rats and the four-plate test in mice) and one exploratory model (the elevated plus-maze test in rats) [56]. Similar as for antidepressive-like behavior, SB-269970 induced anti-anxiety-like effects following a U-shaped dose–response curve in all three models used. So, it appears that inactivation of the 5-HT7 receptor gene does not produce the anti-anxiety phenotype as seen following treatment with SB-269970. This is quite surprising as in several previous studies, SB-269970 consistently mimicked the effects seen in 5-HT7 receptor$^{-/-}$ mice, more specifically with regard to attenuation of thermoregulation [61, 62, 64], anti-immobility in the TST and FST [48, 52], and suppression of REM sleep [52]. While compensatory effects could explain the absence of an anxiolytic-like effect in 5-HT7 knockout mice, it seems very unlikely that the lack of an anxiolytic-like effect as seen in the study of Clemett et al. can be ascribed to compensation mechanisms occurring during the 6-day treatment of rats with 5-HT7 antisense oligonucleotides. An alternative explanation for this remarkable discrepancy could be that anxiolytic-like behavior, in contrast to thermoregulation, depressive-like behavior, and REM sleep, is regulated by a non-G protein-dependent pathway arising from the 5-HT7 receptor, which is inactive in WT animals, but gets stimulated after treatment with SB-269970. Interestingly, in mice, anti-anxiety-like effects are induced by significantly lower doses (0.5 or 1 mg/kg) than dose producing hypothermia, antidepressant-like behavior, and REM sleep suppression (5 or 10 mg/kg). Likewise, the 5-HT1A receptor partial agonist, MM199, evoked an antidepressant-like effect at a dose approximately 16-fold higher than that producing an anxiolytic-like effect in rats [56]. Therefore, it is assumed that the anxiolytic and antidepressant activity of SB-269970 may result from binding to 5-HT7 receptors localized in different brain regions and/or on different neuronal circuitries, exhibiting different affinities for SB-269970. Thus, depressive-like behavior, REM sleep, and thermoregulation could be regulated by a G protein-dependent pathway initiated by 5-HT7 receptors with relatively lower affinities for SB-269970, while anxiety-related behaviors could be regulated by another subpopulation of 5-HT7 receptors displaying higher affinity for SB-269970 and through stimulation of a non-G protein-dependent pathway. As previously mentioned, Wesolowska et al. also showed that intrahippocampal administration of SB-269970 induced a significant antidepressive-like effect in the FST. In addition, intrahippocampal injection of SB-269970 also induced an anxiolytic-like effect in the Vogel conflict drinking test, which, like the anti-immobility effect, also followed a U-shaped dose–response curve. Interestingly, the maximum effect in the Vogel conflict drinking test was observed at a dose of 1 μg SB-269970, whereas the maximum effect in the FST was reached at 3 μg SB-269970 [58]. It is worth noting here that, as previously mentioned, treatment with SB-269970 as well as knockout of the 5-HT7 receptor gene reduced marble burying behavior, which can also be considered as an anxiolytic effect. Interestingly, this effect was seen at a relatively high dose of SB-269970, namely 10 mg/kg [66].

## Schizophrenia

The 5-HT7 receptor has already early been implicated in schizophrenia, as a large number of both typical and atypical antipsychotic drugs were shown to have high affinity for the 5-HT7 receptor. So at least some of their effects could be mediated by the 5-HT7 receptor [134].

Schizophrenia is a multifaceted syndrome, and so, it is unlikely that an animal model can be created for this complex mental disorder. However, much energy has been devoted to the development of models of specific dysfunctions characterizing schizophrenic patients. Together with Huntington's disease, Tourette's syndrome, and obsessive–compulsive disorder, schizophrenia is considered as a "gating disorder", as schizophrenic patients also exhibit deficits in "sensorimotor gating". This implies that sensory stimuli are not properly "gated" or filtered out, leading to a chaotic flow of information reaching consciousness, which is experienced as overwhelming. An operational measure for deficient sensorimotor gating is the loss of normal "prepulse inhibition" (PPI), which is "the normal inhibition of a startle reflex when the startling stimulus is preceded by

a weak prestimulus". In rodents, disruption of PPI can be induced pharmacologically by stimulation of D2 dopamine receptors with amphetamine or apomorphine, activation of serotonergic systems by agonists at multiple 5-HT receptors, or blockade of NMDA receptors with drugs, such as phencyclidine (PCP). In addition, some developmental manipulations, such as isolation rearing, also lead to deficits in PPI in rodents. So, four main animal models of disrupted PPI have been developed, of which three pharmacological (a dopaminergic, serotonergic, and glutamatergic model) and one non-pharmacological (the isolation rearing model), and these all have been applied for the evaluation of potential antipsychotics. The dopaminergic PPI model appeared to be sensitive to both typical and atypical antipsychotics, whereas deficits in PPI produced by NMDA antagonists appear to be more sensitive to clozapine-like atypical antipsychotics than to typical antipsychotics [135].

In order to evaluate the role of the 5-HT7 receptor in disruption of PPI, Pouzet et al. tested the influence of the selective 5-HT7 receptor antagonist, SB-258741, in two models of PPI impairment in Wistar rats. SB-258741 did not affect amphetamine-induced disruption of PPI, while PCP-induced PPI deficits were counteracted by SB-258741 [70]. In agreement with this, the ability of apomorphine and amphetamine to disrupt PPI was unaltered in 5-HT7 receptor$^{-/-}$ mice, whereas disruption of PPI by PCP was diminished in the 5-HT7 receptor$^{-/-}$ mice compared to 5-HT7$^{+/+}$ mice [136]. So, the PPI deficits produced by inhibition of the NMDA receptor seem to be dependent on 5-HT7 receptor activation, which suggests that the NMDA receptor exerts a negative influence on serotonergic neurons and subsequent activation of the 5-HT7 receptor. This is in contrast with the observations of Harsing et al., showing that both NMDA and AMPA stimulate 5-HT release in the DRN [137]. On the other hand, dopaminergic regulation of PPI seems to occur independent of 5-HT7 receptor activation. However, in the same study by Semenova et al., SB-269970 did not influence PCP-induced PPI deficits in either C57BL/6J mice or Wistar rats [136]. Moreover, in a recent study, using C57BL/6 mice, SB-269970 was able to reverse amphetamine-induced disruption of PPI, while again no effect of SB-269970 was seen on the glutamatergic component of PPI, as evaluated by ketamine [71].

So, apparently, SB-269970 produces an opposite phenotype as seen after induction with SB-258741 and in 5-HT7 receptor knockout mice. As SB-269970 and SB-258741 display full and partial inverse agonist activity, respectively, at the 5-HT7 receptor with regard to $G_s$-dependent signaling [138], it is quite surprising that these antagonists induce opposite phenotypes. Similar as for anxiety, these discrepancies could be explained by the fact that impairment of PPI is regulated by a non-G protein-dependent pathway arising from the 5-HT7 receptor, which is stimulated by SB-269970, but inhibited by SB-258741. The attenuation of PPI impairment in response to dopaminergic signaling after treatment with SB-269970 might reflect an inhibitory influence of the 5-HT7 receptor on this dopaminergic signaling, through a G protein-independent pathway, triggered by SB-269970. So, despite having very similar chemical structures [126], SB-258741 and SB-269970 seem to have significantly different biological activities.

Additional evidence supporting a role of the 5-HT7 receptor in schizophrenia comes from a postmortem study, showing a decrease of 5-HT7 receptor mRNA in the dorsolateral prefrontal cortex of schizophrenics [139], a region implicated in this disorder. In line with this, Dean et al. observed a significant decrease of [$^3$H]SB-269970-binding sites in Brodmann's area 9—a part of the dorsolateral prefrontal cortex—in postmortem tissue of subjects with schizophrenia [140]. The observed decrease of 5-HT7 receptor both at the mRNA and protein level could point to long-term downregulation of this receptor following chronic overstimulation in schizophrenic patients. As the 5-HT7 receptor has been shown to play an important role in the regulation of cortical synaptic activity [141], this receptor could contribute to the abnormal functioning of the prefrontal cortex followed by cognitive deficits seen in schizophrenic patients. In line with this, deficits in rat reversal learning induced by PCP, which is a behavioral model for the cognitive deficits associated with schizophrenia, is attenuated by SB-269970 [72]. East et al. also showed that a 2-week treatment of rats with antipsychotics did not have an effect on the 5-HT7 receptor mRNA level in the cortex [139], while haloperidol treatment for 1 month produced an increase in [$^3$H]SB-269970-binding sites in the rat cortex [140]. So, antipsychotic treatment possibly inhibits 5-HT7 receptor activation, thereby restoring normal membrane expression of this receptor.

In order to evaluate whether certain 5-HT7 receptor gene variants are associated with schizophrenia, Ikeda et al. selected four 5-HT7 receptor single nucleotide polymorphisms (SNPs) for a case–control association analysis in 383 Japanese schizophrenia patients and 351 controls. Two SNPs, an intronic SNP (SNP5) and a promoter SNP (SNP2), showed an association with schizophrenia, but SNP2 was not found to have any functional relevance [142]. Interestingly, previous genome-wide linkage studies of schizophrenia also showed a linkage in 10q22 with schizophrenia, which is close to the location of the human 5-HT7 receptor gene (10q21–24) [143, 144].

Finally, risperidone is an atypical antipsychotic, which has several advantages compared to typical antipsychotics, including a lower incidence of extrapyramidal

side effects, a more frequent clinically significant improvement, and a broader-ranging effect on both positive and negative symptoms of schizophrenia. However, there are still remarkable individual differences in the response to this antipsychotic, and this has been shown to be greatly influenced by genetic factors. As risperidone has been shown to pseudo-irreversibly bind and inactivate the human 5-HT7 receptor [145], an association study was performed between polymorphisms found in the 5-HT7 receptor gene and therapeutic efficacy of risperidone [146]. Unfortunately, no significant correlation was detected. Interestingly, Knight et al. extended the study of Smith et al. [145] and found that maximal concentrations of both risperidone and 9-OH-risperidone, which is the active metabolite of risperidone, completely inhibited all the functional activity of the 5-HT7 receptors but occupied only 50% of the 5-HT7 receptors [147]. In other words, 50% of the 5-HT7 receptors seemed to be risperidone- and 9-OH-risperidone-resistant, which led the authors to suggest the involvement of dimerization. One possibility is that binding of risperidone or 9-OH-risperidone to one protomer of a dimer pair produces an allosteric effect on the other protomer, which loses its ability to bind the antagonists.

## Epilepsy

A role for the 5-HT7 receptor in seizure disorders was initially suggested when a significant correlation was found between the affinities of several non-specific antagonistic compounds for the 5-HT7 receptor and their ability to protect mice against audiogenic seizures [148]. More recently, the selective 5-HT7 receptor antagonist, SB-258719, was shown to significantly decrease the number of paroxysms and the duration of spike-wave discharges in the WAG/Rij rat model of absence epilepsy [73]. Autoradiographic mapping also showed that 5-HT7 receptor-binding sites were particularly enriched in the thalamus [45], which has been implicated in the generation of spike-wave discharges [73]. Surprisingly, constitutive deletion of 5-HT7 receptors was found to generally reduce the threshold for seizures in mice [74]. Seizures were produced by three types of electrical stimulation and three chemical convulsants and were compared in 5-HT7 KO mice and WT mice. The thresholds of electroshock-induced tonic seizures were significantly lower in KO than in WT mice, whereas the thresholds of electroshock-induced clonic and limbic seizures were the same. In addition, pentylenetetrazole and cocaine were more potent in inducing seizures in 5-HT7 KO mice than in WT mice. So, despite the fact that different epileptic animal models were used, SB-258719 appeared to be anticonvulsant, while knockout of the 5-HT7 receptor had a proconvulsant effect. As for anxiety and schizophrenia, these opposing effects of 5-HT7 receptor antagonism and 5-HT7 receptor knockout on epileptic seizures could be explained by the involvement of a non-G protein-dependent pathway arising at the 5-HT7 receptor, which is stimulated by SB-258719. This implies that SB-258719 also would exhibit biased activity toward the 5-HT7 receptor, as has been suggested earlier for SB-269970.

## Migraine

The 5-HT7 receptor was quickly associated with migraine, as most of the migraine prophylactic drugs, such as amitriptyline, chlorpromazine, cyproheptadine, lisuride, LY215840, metergoline, methysergide, mianserin, and sergolexole, display relatively high affinity for the recombinant 5-HT7 receptor. Most interesting is the observation that the average pharmaceutically active doses of several migraine prophylactic drugs, including amitriptyline, chlorpromazine, cyproheptadine, lisuride, methysergide, and mianserin, correlate significantly with their reported affinity at the recombinant 5-HT7 receptor. Based on these findings, it seems reasonable to hypothesize that the migraine prophylactic efficacy of these antagonists is due to, at least in part, blockade of 5-HT7 receptors.

According to the vascular hypothesis of migraine, cerebrovascular dilatation induced by 5-HT release in response to stress underlies the development of headache. Interestingly, early transcranial Doppler sonography studies showed a significant decrease in middle cerebral artery blood velocity on the headache side in migraine patients, whereas blood flow velocity was unchanged in the non-headache side [149]. Furthermore, some of the abovementioned migraine prophylactic drugs, i.e., lisuride, LY215840, metergoline, methysergide, mianserin, and sergolexole, had been shown to antagonize 5-HT receptor-induced vasorelaxation in several vascular smooth muscle preparations, including the external carotid circulation [150], which has long been suggested to be involved in the pathophysiology of migraine. Based upon the rank order of agonist potencies (5-CT >> 5-HT ≥ 5-methoxytryptamine) and the antagonism of a series of drugs showing high affinity for the cloned 5-HT7 receptor (methiothepin, mesulergine, metergoline, methysergide, lisuride, and clozapine), Villalon et al. indicated that the 5-HT receptor, mediating external carotid vasodilatation in GR-127935-pretreated vagosympathectomized dogs, is operationally similar to the 5-HT7 receptor [150]. Similarly, Terron and Falcon-Neri showed by means of

pharmacological profiling that the 5-HT receptor, eliciting direct relaxation in canine basilar and middle cerebral arteries, highly resembles the 5-HT7 receptor [151]. Interestingly, endothelium-denuded preparations were employed in the latter study, suggesting that the relaxant effect of the putative 5-HT7 receptor is most likely due to an action on smooth muscle cells. Moreover, the relaxant response could not be inhibited by pretreatment with a nitric oxide synthase or cyclooxygenase inhibitor. In a similar way, Ishine et al. showed that the 5-HT inhibition of porcine pial venous rhythmic contractions and relaxation is mediated at least in part by 5-HT7 receptors located on smooth muscle cells [152]. In this regard, pig cerebral arterial smooth muscle had also been shown to contain 5-HT7 receptor mRNA [153]. Among the various cranial vessels implicated in migraine headache, the middle meningeal artery has been considered as one of the most important for several reasons: It is the largest artery supplying the dura mater, its stimulation leads to activation of second-order trigeminal nociceptive pathways in the brain stem of mammals, and its stimulation is pain producing in humans. It is in this artery that Terron and Martinez-Garcia showed that 5-CT-induced hypotensive responses in anesthetized rats were strongly inhibited by specific blockade of the 5-HT7 receptor with SB-269970 [78]. This provides additional support to the notion that 5-HT promotes cranial dilatation and migraine via activation of 5-HT7 receptors.

Finally, a common neurochemical feature of migraine and other stress-related disorders, including anxiety and depression, is a dysfunction of the central serotonergic system, involving abnormally low levels of 5-HT in the brain. It has been hypothesized that this reduction of central 5-HT levels between attacks leads to sensitization of craniovascular 5-HT7 receptors, which causes an exaggerated vascular dilatation upon release of 5-HT during migraine attacks [154]. To investigate this "sensitization hypothesis", Martinez-Garcia et al. studied the effect of experimental reduction of brain 5-HT levels—by pretreatment with the neurotoxin 5,7-dihydroxytryptamine (5,7-DHT)—on 5-CT-induced vasomotor responses in the middle meningeal artery of anesthetized rats. However, the meningeal dilator responses produced by 5-CT were almost identical in vehicle- and 5,7-DHT-pretreated animals, suggesting that a reduction of 5-HT levels in the brain does not promote 5-HT7 receptor sensitization in the middle meningeal artery [79]. In support of a major role for 5-HT7 receptors in 5-CT-induced dilatation, SB-269970 strongly inhibited the hypotensive and meningeal dilator responses produced by this agonist in both vehicle- and 5,7-DHT-pretreated rats, which closely resembled the effect of the antagonist seen in intact anesthetized rats [78].

## Sensation-Seeking and Impulsive Behavior

Sensation-seeking behavior in humans is a personality trait characterized by voluntary participation in activities involving personal risk and is associated with a greater propensity to use psychoactive substances. A reliable animal model of this human trait, which also shows high predictability of drug use, is based on the variety of behavioral responses that rats exhibit after forced exposure to a novel and inescapable environment. This model categorizes animals into two groups: high responders (HR), being highly active, and low responders (LR), showing less exploration. Interestingly, HR rats also clearly show increased drug-taking and decreased anxiety-like behavior in comparison to LR rats. There is evidence that the individual differences in novelty seeking behavior may be caused by differential 5-HT-mediated neurotransmission. In order to investigate whether the 5-HT7 receptor plays a role in this, Ballaz et al. compared gene expression of this receptor in the brains of HR and LR rats by means of in situ hybridization histochemistry. Levels of 5-HT7 transcripts appeared to be significantly lower in HR rats than in LR rats, predominantly in neural areas such as the intralaminar and paraventricular thalamic nuclei, as well as the dorsal hippocampus [155]. Interestingly, the hippocampal 5-HT7 receptor has been shown to influence the HPA axis [156, 157], which appears to be more active following novelty-triggered stress in HR rats than in LR rats. So, the relatively high levels of 5-HT7 receptor in the hippocampus of LR rats may negatively affect the HPA axis, more specifically decrease the levels of circulating corticosteroids and stimulate GR expression in the hippocampus, and in that way diminish exploration of a novel stress-inducing environment. It has to be noted here that the paraventricular nucleus of the hypothalamus is in fact a key element of the HPA axis, containing neuroendocrine neurons that synthesize and secrete vasopressin and corticotrophin-releasing hormone. So, besides hippocampal 5-HT7 receptors, the 5-HT7 receptor in the paraventricular nucleus may also dampen novelty seeking in a similar way, namely through attenuation of the HPA axis. In a following study by Ballaz et al., HR and LR rats were tested in a novel object discrimination (NOD) test in order to compare their attention and memory. The test requires the animal to retrieve memories of a previously encountered object ("old or sample object") after a delay of 3 h and discriminate it from a novel object. LR rats spent significantly less time exploring the old (sample) object than the novel object, whereas HR rats spent an equal amount of time exploring the new and old (sample) object. So, in contrast to HR rats, which did not recognize the old object and treated it as new, LR rats were able to recognize a previously encountered object and discriminate it from a new object. To verify

whether the observed decrease in attention and memory in HR rats was caused by the lower 5-HT7 receptor expression level, both HR rats and LR rats were treated with SB-269970 before the start of the trial. SB-269970 did not affect the exploration times of HR rats but curtailed the differences between the exploration times in LR rats. Surprisingly, this equalization of the exploration times in LR rats was not the result of an increased exploration or, in other words, a lack of recognition of the old object but was caused by a significant decrease of the exploration time of the new object. So, while not affecting the episodic memory in this NOD task, SB-269970 clearly influenced the adaptive behavior of the LR rats in response to a novel object in the environment. SB-269970 did not decrease the exploration times of HR rats, and so, the low levels of 5-HT7 receptors present in these rats are not involved in their adaptive behavior to new objects. In conclusion, inhibition of the 5-HT7 receptor on itself is not sufficient to impair episodic memory in this NOD task but significantly affected the adaptive behavior of LR rats in response to a change in the environment [80].

Drug abuse is not only correlated with novelty seeking behavior but also with impulsivity, which has been defined as following: "a predisposition toward rapid, unplanned reactions to internal or external stimuli without regard to the negative consequences of these reactions to themselves or others" [158]. Individuals with substance abuse have been shown to have higher impulsivity than non-substance-using populations, and children and adolescents, who have the highest rates of later substance abuse, also show increased impulsivity. Moreover, impulsivity appears to have a negative impact on substance abuse treatment [159]. Leo et al. showed that methylphenidate (MPH) administration to adolescent rodents produced a marked increment of 5-HT7 receptor expression and synaptic contacts, mainly in the nucleus accumbens, and a reduction of basal behavioral impulsivity. Administration of SB-269970 fully counteracts the MPH-reduced impulsive behavior and also enhances impulsivity when administered alone in naive rats. On the contrary, 8-OH-DPAT reduces impulsive behavior in naive adolescent and adult rats. In summary, these behavioral pharmacology experiments clearly show that the 5-HT7 receptor mediates self-control behavior [81]. Interestingly, Adriani et al. showed that the 5-HT7 receptor mRNA was also upregulated in the striatum following MPH treatment [160]. In this regard, Leo et al. showed that activation of the 5-HT7 receptor significantly increased neurite length in striatal neuron primary cultures [81], which suggests a role of the 5-HT7 receptor in plastic remodeling of neuronal morphology. So, the 5-HT7 receptor could suppress impulsive behavior by promoting neuronal differentiation in the striatum, among others.

## Memory

From *Aplysia* to human studies, evidence exists that the 5-HT system mediates learning and memory processes. In *Aplysia*, 5-HT has been shown to play a role in memory through the activation of a cAMP-PKA-dependent pathway within specific sensory neurons. Nevertheless, most data derive from studies on mammalian species, including man, which indicate the involvement of 5-HT in both short-term (STM) and long-term memory (LTM). Moreover, a great deal of evidence has underlined the involvement of 5-HT7 receptors in memory mechanisms. In a recent study, Gasbarri et al. assessed the role of the 5-HT7 receptor on both working and reference memory in a radial arm maze task using the selective 5-HT7 receptor antagonist, SB-269970. This task consists of an acquisition phase and a test phase, which are separated by a delay of varying duration, ranging from 20 min to 4 h, and serve to evaluate working and reference memory, respectively. SB-269970 decreased the number of errors in the test phase and thus positively affected reference memory, while no effects were seen on working memory in the acquisition phase [82]. It should be noted here that SB-269970 only improved reference memory when administered before the acquisition phase, but not when administered immediately after the acquisition phase or just before the test phase. Nevertheless, we can conclude that endogenous 5-HT7 receptor activity during the acquisition phase attenuates reference memory formation. In contrast, in a Pavlovian/instrumental autoshaping learning task, in which the test session took place 24 h after the training session, administration of 8-OH-DPAT immediately after the training session facilitated memory consolidation. Co-administration of the selective 5-HT1A receptor antagonist, WAY-100635, as well as the 5-HT7 receptor antagonists, SB-269970 and DR-4004, significantly inhibited the facilitatory 8-OH-DPAT effect [84, 85]. Post-training administration of WAY-100635 or DR-4004 alone, however, had no effect on autoshaping [84]. In a subsequent study, Perez-Garcia and Meneses demonstrated that post-training administration of the 5-HT7 receptor agonist, AS-19, likewise enhanced memory formation in the autoshaping Pavlovian/instrumental learning task, an effect that was reversed by SB-269970, but not by WAY-100635 [86]. All together, these data clearly confirm a facilitatory role for both the 5-HT1A and 5-HT7 receptor in long-term memory formation. More recently, Meneses et al. studied the role of the 5-HT7 receptor in both STM and LTM using an associative learning task, in which test sessions took place 1.5 (STM) and 24 h (LTM) after the training session, or only 24 h later to asses memory consolidation. Interestingly, post-training administration of AS-19 impaired STM but improved both LTM and memory consolidation. SB-269970 reversed the AS-19-induced

STM amnesic effect and the AS-19-SB-269970 combination impaired LTM, while SB-269970 alone had no effect [83]. In short, all the abovementioned data indicate that the 5-HT7 receptor has opposite effects on STM, LTM, and memory consolidation, attenuating STM, while enhancing LTM and memory consolidation. Additionally, SB-269970, DR-4004, as well as AS-19 were all able to reverse amnesia induced by post-training administration of scopolamine (a cholinergic antagonist) or dizocilpine (a non-competitive NMDA receptor antagonist) [85, 86]. This supports the hypothesis that cholinergic, glutamatergic, and serotonergic systems interact in cognitively impaired animals. Moreover, it shows that the 5-HT7 receptor can significantly influence cognitive dysfunction, and therefore, this receptor could represent a potential therapeutic target for the treatment of memory dysfunction, as seen in cognitive disorders, such as schizophrenia and Alzheimer's disease, or age-related decline.

In a study by Roberts et al., the role of the 5-HT7 receptor was studied in five types of learning by means of 5-HT7 receptor knockout mice. Two forms of place learning were studied, namely spatial learning in the Barnes maze test and contextual fear conditioning. In place learning, integration of polymodal contextual information is important, which is known to be mediated by the hippocampus. In addition, three hippocampus-independent learning tests were performed: motor learning, cued conditioning, and operant conditioning. 5-HT7$^{-/-}$ mice exhibited normal motor and spatial learning ability, as well as normal cued and operant conditioning, but showed a selective impairment in contextual fear conditioning. The dissociation between contextual-based learning and cued food conditioning indicates that the 5-HT7 receptor is only needed for the more complex integrative learning mechanisms involved in contextual experiments. However, a lack of impairment in the spatial Barnes maze task suggests that the 5-HT7 receptor is not critical for place learning in general [69]. In order to detect possible neuronal mechanisms underlying the observed learning deficit, Roberts et al. studied synaptic plasticity within the CA1 region of the hippocampus, which is known to contain 5-HT7 receptor-binding sites [161]. Using paired pulse facilitation and paired pulse inhibition, it was observed that short-term plasticity was not altered in the 5-HT7$^{-/-}$ mice. On the contrary, a reduced long-term potentiation was seen in hippocampal slice preparations derived from 5-HT7$^{-/-}$ mice compared to 5-HT7$^{+/+}$ mice [69]. In conclusion, the 5-HT7 receptor is clearly of importance for contextual hippocampal-dependent learning, for which a possible neuronal correlate is present within the CA1 region of the hippocampus. In this regard, 8-OH-DPAT was previously shown to impair aversive contextual learning in the passive avoidance task in mice, which was attenuated by several 5-HT1A receptor antagonists [162]. However, SB-269970 combined with 8-OH-DPAT resulted in an intensification of the 8-OH-DPAT-induced impairments, indicating that 8-OH-DPAT-mediated stimulation of 5-HT7 receptors counteracts the impairing effects mediated primarily via 5-HT1A receptors. Treatment with SB-269970 alone had no effect in the passive avoidance task, which implies that basal 5-HT7 receptor activity is rather low and plays a rather marginal role during acquisition of passive avoidance learning [87]. Finally, in order to study the role of the 5-HT7 receptor in hippocampus-dependent learning and memory, Sarkisyan and Hedlund studied the effect of pharmacological blockage as well as inactivation of the 5-HT7 receptor in the following behavioral models: novel object test, novel location test, and Barnes maze test. Both 5-HT7$^{+/+}$ and 5-HT7$^{-/-}$ mice performed a comparable object novelty recognition, as did vehicle- and SB-269970-treated mice. In the novel location test, animals lacking the 5-HT7 receptor, however, exhibited reduced sensitivity to spatial changes in their environment compared to their wild-type siblings. A similar effect was seen after treatment with SB-269970 [125]. To test additional spatial memory, Sarkisyan and Hedlund performed a modified Barnes maze test, in which the acquisition phase was followed by a probe test, a retention test, and a reversal test. In the Barnes maze test, the mouse escapes bright light and noise by entering a tunnel (the escape box) under one of 20 holes around the edge of a circular platform. In the study of Sarkisyan and Hedlund, the acquisition phase consisted of 12 sessions and was followed by a 13th session, being the probe test, in which the escape box was removed. One month later, the animals were tested in a regular setting—with the escape box back at its original location—for memory retention. The following day a final session was performed, in which the escape box was moved to a new location, 180° from its original position (reversal test). The 5-HT7$^{-/-}$ mice performed virtually identically to their 5-HT7$^{+/+}$ siblings during the 12 initial sessions, indicating that the 5-HT7$^{-/-}$ mice did not exhibit learning impairments and/or dysfunctions in short-term spatial memory. Both genotypes could also efficiently locate the escape box during the retention test, and so, the 5-HT7$^{-/-}$ mice did not seem to have impairments in long-term memory nor memory consolidation compared with their wild-type siblings. In both the reversal and probe test, the 5-HT7$^{+/+}$ mice spent significantly less time in the original quadrant of the escape box. More specifically, both genotypes initially followed the same direct path toward the presumed position of the escape box, and when reaching the empty escape hole, they both began exploring the maze. However, when the 5-HT7$^{-/-}$ mice happened to pass the vicinity of the starting position, these mice took the initial route back to the empty escape box. A possible explanation for this could be the

reliance of 5-HT7$^{-/-}$ mice on a mainly striatum-dependent egocentric memory, which is characterized by a passive form of information processing. In contrast, the 5-HT7$^{+/+}$ mice exhibited less backtracking compared with the 5-HT7$^{-/-}$ mice, as their search strategy more likely involved hippocampus-dependent allocentric spatial memory, which is a more continuous and active information processing. So, it appears that the switch from a striatal strategy toward hippocampus-dependent allocentric memory is impaired in 5-HT7$^{-/-}$ mice [125]. A unique feature of the hippocampus is its ability to generate new neurons, even in adulthood, which have been shown to become synaptically active [163]. Moreover, it has been suggested that the rate of adult neurogenesis is linked with memory consolidation and spatial learning [164]. As knockout of the 5-HT7 receptor specifically induced a deficit in hippocampus-dependent allocentric spatial memory, it was investigated whether this was correlated with an overall decrease in hippocampal cell proliferation. Therefore, the number of proliferating cells was determined in both 5-HT7$^{+/+}$ and 5-HT7$^{-/-}$ mice by counting the number of cells that incorporated 5′-bromo-2′-deoxyuridine in the subgranular zone of the dentate gyrus. Unfortunately, no difference could be observed in cell proliferation between 5-HT7$^{+/+}$ and 5-HT7$^{-/-}$ mice, which is actually in agreement with the unaltered spatial learning, memory consolidation, and memory retrieval seen in 5-HT7$^{-/-}$ mice. Nevertheless, the 5-HT7 receptor could still be involved in cell differentiation and/or synaptic plasticity rather than cell proliferation [125].

## Pain

Rocha-Gonzales et al. studied the role of both peripheral and spinal 5-HT7 receptors in nociception in rats by means of the formalin test, in which formalin is injected into the paw and induces flinching behavior. Therefore, micro-injection of formalin was preceded by either local or spinal administration of SB-269970 and/or 5-HT and 5-CT. Local administration of SB-269970 significantly reduced 1% formalin-induced flinching, while local 5-HT or 5-CT augmented dose-dependently 0.5% formalin-induced nociceptive behavior, which was significantly reduced by SB-269970 [165]. So, activation of the 5-HT7 receptor in the paw induces nociceptive behavior and also mediates formalin-induced nociception. In agreement with this, immunostaining showed the presence of 5-HT7 receptors in myelinated and unmyelinated axons of the digital nerves in rat hindpaw. Administration of SB-269970 into the spinal cord, however, did not affect nociceptive behavior in response to formalin. Nevertheless, low doses of intrathecal (i.t.) 5-CT increased formalin-induced flinching, which was reduced by SB-269970 [165]. So, although 5-HT7 receptor activation in the spinal cord is not necessary for formalin-induced nociception, it potentiates the latter in the presence of low levels 5-CT. In agreement with this, 5-HT7 receptor knockout mice showed identical nociceptive behavior as wild-type mice in the tail-flick test [69]. It should be noted here that high levels of i.t. administered 5-CT, in contrast to low 5-CT levels, decreased formalin-induced nociceptive behavior, which was partially inhibited by the 5-HT1A receptor antagonist, WAY-100635. Unfortunately, the effect of SB-269970 was not mentioned here, so it is unclear if this antinociception is also mediated by spinal 5-HT7 receptors. In this regard, Dogrul and Seyrek showed that in mice, morphine-induced antinociception, as assessed by the radiant heat tail-flick test, is completely blocked by i.t. administered SB-269970 [166]. Similarly, spinal administration of SB-269970 in rats markedly attenuated the antinociceptive effects of morphine in the thermal paw-flick test [167]. Very recently, the antinociceptive and antihyperalgesic effects of tramadol and its major active metabolite *O*-desmethyltramadol in mice were blocked by i.t. injection of both SB-269970 and SB-258719 in the radiant heat tail-flick and plantar incision test [75]. These studies indicate that activation of spinal 5-HT7 receptors plays a key role in the antinociceptive pathway activated by both morphine and tramadol. Therefore, it could be that spinal 5-HT7 receptors also exhibit biphasic activity, promoting and inhibiting nociception in the presence of low and high levels of 5-HT, respectively. Interestingly, immunocytochemical studies at the lumbar level of the spinal cord found that 5-HT7 receptors are mainly localized in the two superficial laminae of the dorsal horn and in small- and medium-sized DRG cells, which is consistent with a predominant role in nociception [168, 169]. Electron microscopic examination of the dorsal horn further revealed three main localizations: (1) postsynaptic localization on peptidergic cell bodies and in numerous dendrites, (2) presynaptic localization on unmyelinated and thin myelinated peptidergic fibers, and (3) on astrocytes.

Besides peripheral and spinal 5-HT7 receptors, supraspinal 5-HT7 receptors have also been implicated in the modulation of nociception. Intracerebroventricular administration of the 5-HT1/5-HT2/5-HT7 receptor antagonist, methiothepin, was shown to significantly inhibit the antinociceptive effect of the non-steroidal anti-inflammatory drug, S-(+)-ketoprofen, as assessed by the pain-induced functional impairment model in the rat [170]. Shortly thereafter, administration of 8-OH-DPAT into the medial thalamus, more specifically the nucleus parafascicularis and central lateral thalamic nucleus, was shown to raise the threshold of the electrical tailshock intensity to trigger vocalizations, an antinociceptive effect that was blocked by intrathalamic micro-injection of SB-269970 [76]. Interestingly, 5-HT7 receptor activation in the thalamus only had an

antinociceptive effect on vocalizations, which are pain behaviors organized at the medullary and forebrain level of the neuraxis, but did not suppress the spinal motor reflexes, which are triggered at the spinal level.

In order to further examine the nociceptive role of the 5-HT7 receptor, Brenchat et al. studied the influence of systemically administered 5-HT7 receptor agonists and antagonists on capsaicin-induced mechanical hypersensitivity in mice. All the agonists used, namely E-55888, AS-19, and MSD-5a, dose-dependently inhibited capsaicin-induced mechanical hypersensitivity, which was prevented by both 5-HT7 receptor antagonists, SB-269970 and SB-258719. The order of efficacy was E-55888 > AS-19 > MSD-5a and matched their in vitro efficacy at the 5-HT7 receptor [77]. On the other hand, SB-269970 and SB-258719 both exerted a dose-dependent pronociceptive effect, promoting mechanical hypersensitivity in mice, injected with a low subactive dose of capsaicin, but not with vehicle. This indicates that antinociceptive activity of the 5-HT7 receptor only takes place after challenge with capsaicin, but not with vehicle, thereby allowing the antagonists to exert their counteracting pronociceptive effect [77]. As systemic administration of 5-HT7 receptor agonists in this animal model of nociception clearly has an analgesic effect, 5-HT7 receptor agonists may represent new potential therapeutics for pain alleviation.

## Discussion and Conclusion

The use of both pharmacological and genetic tools has increased our knowledge of the 5-HT7 receptor substantially. Today it is clear that the 5-HT7 receptor is involved in a variety of CNS functions: circadian rhythm, REM sleep, depression, thermoregulation, OCD, anxiety, schizophrenia, epilepsy, nociception, migraine, sensation-seeking and impulsive behavior, and memory. For some of these CNS functions, however, results obtained with 5-HT7 receptor knockout mice, selective antagonists and agonists, were quite contradictory. In this review, we extensively discussed the role of the 5-HT7 receptor in the above-mentioned CNS functions and also proposed some hypothetical models, which could explain the observed inconsistencies. More specifically, both REM sleep and depressive(-like) behavior seem to be attenuated by stimulation as well as inhibition of 5-HT7 receptor activity. As for nociception, activation of the 5-HT7 receptor at the level of the spinal cord appears to induce both pro- and antinociceptive effects. These seemingly conflicting results led to the suggestion that REM sleep, depressive(-like) behavior, and nociception could be regulated by the 5-HT7 receptor in a biphasic way, with high and low levels of 5-HT inducing opposite signaling pathways. In preclinical animal models for anxiety, schizophrenia, and epilepsy, knockout of the 5-HT7 receptor induced a different, sometimes even opposite, phenotype than seen after treatment with certain 5-HT7 receptor antagonists (SB-269970 and SB-258719). This is usually ascribed to compensatory mechanisms following genetic activation of the 5-HT7 receptor, and although this could be possible, we suggest an alternative explanation. That is to say, anxiety, schizophrenia, and epilepsy could be regulated by a non-G protein-dependent pathway arising at the 5-HT7 receptor, which could get stimulated rather than inhibited by SB-269970 or SB-258719. This implies that SB-269970 and SB-258719, in contrast to SB-258741, would exhibit biased activity toward the 5-HT7 receptor, inhibiting G protein-dependent signaling, while activating one or more G protein-independent pathways. Interestingly, both biphasic signaling and G protein-independent signaling have been shown to be mediated by GPCR dimerization. Therefore, we propose a model, in which the 5-HT7 receptor resides in different dimeric contexts and initiates different signaling pathways, depending on the neuronal circuitry and/or brain region. In other words, GPCR dimerization could be the key mechanism that brings diversity into 5-HT7 receptor signaling. This dimer-based diversification of 5-HT7 receptor signaling would also explain how one single receptor can be involved in so many CNS functions. As the 5-HT7 receptor most probably assumes different conformations depending on its dimeric state, it should be possible to develop therapeutics that specifically target the 5-HT7 receptor in a certain dimeric state. These so-called dimer-specific therapeutics would then affect 5-HT7 receptor signaling only in a certain neuronal circuitry and/or brain region and hence would act more specifically and display fewer side effects. In addition to dimer specificity, therapeutical ligands can also exhibit pathway specificity, which is a relatively new concept within the GPCR field and is referred to as ligand bias. This implicates that ligands selectively influence a subset of signaling pathways that arise at a given GPCR; for example, a ligand can inhibit G protein signaling and at the same time stimulate β-arrestin-dependent signaling.

In conclusion, the use of 5-HT7 receptor knockout mice, selective antagonists and agonists, have proven very useful in the elucidation of CNS functions, in which the 5-HT7 receptor is involved. For future research of the 5-HT7 receptor and even of GPCRs in general, we highlight GPCR dimerization and G protein-independent signaling as two promising directions, which should give further insight in how distinct CNS functions are differentially regulated by the 5-HT7 receptor. Obviously, the study of these concepts should be done as much as possible in native brain populations in their natural environment. Ultimately, the gained insights might lead to the development of more efficient dimer- and/or pathway-specific therapeutics.

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