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Review

Unraveling the Involvement of Serotonergic 5-Hydroxytryptamine Receptor-6 Activation in Chronic Pain: A Narrative Review

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Abstract

Chronic pain is a significant and global healthcare issue that hugely implies the quality of life and productivity of the affected individuals. It is challenging to treat and thus necessitates a deeper understanding of its underlying pathomechanisms to develop targeted interventions. Serotonin is one of the essential neurotransmitters involved in the propagation of pain signals through both ascending and descending pathways, acting via various receptor subtypes, including the 5-hydroxytryptamine receptor-6 (5-HT_6R). Recent studies have shed light on the involvement of 5-HT_6R in the pathophysiology of chronic pain. This review aimed to uncover the emerging roles of 5-HT_6R in chronic pain research by focusing on its functions in pain

modulation, neuronal excitability, and pain processing. Although 5-HT_6R has been recently discovered, previous studies have revealed its therapeutic effects in modulating chronic pain. Understanding the precise mechanism underlying the 5-HT_6R may offer new avenues for future strategies of chronic pain management and indirectly improve the individual's quality of life. However, further research is warranted to elucidate the intricate interplay between 5-HT_6R and other pain modulatory pathways, paving the way for more effective and tailored therapeutic strategies for chronic pain.

Keywords
5-hydroxytryptamine-6 receptor; serotoninergic receptor; chronic pain; pain physiology

1. Introduction

Chronic pain is a global public health concern. The International Association for the Study of Pain reported that approximately 5-10% of individuals suffered from chronic pain of neuropathic origin resulting from abnormalities or lesions of the central or peripheral somatosensory nervous system [1]. Although the pain itself does not cause immediate death, the prolonged suffering from pain negatively impacts the career and quality of life of the affected individuals. In clinical practice, first-line medications for peripheral neuropathic pain, such as tricyclic anti-depressants, gabapentin, and topical drugs, provide only partial effectiveness. According to the number needed to treat (NNT) metric, between 3.6 and 7.7 patients must be treated for one patient to achieve a 50% reduction in pain [2]. Chronic pain is defined as pain that lasts for several weeks or longer even after the key causative elements have receded. It is categorized into neuropathic pain (i.e., pain that is derived from nerve injury) and nociceptive pain (i.e., pain caused by ongoing inflammation and non-neuronal tissue injury) [3]. Unfortunately, while acute pain possesses protective value, chronic pain is more of a pathological disorder with no apparent health benefits. It frequently results in major mental illnesses such as chronic anxiety, depression, learning, and memory impairment [4]. Thus, understanding the deeper mechanisms involving chronic pain, especially neuropathic pain, is critical to alleviating the symptoms and discovering the potential targets for the analgesic purpose.

Serotonin, also known as 5-hydroxytryptamine (5-HT) is an essential neurotransmitter involved in the regulation of various fundamental roles of peripheral and central nervous systems (PNS and CNS, respectively). Nearly all the neurons in the CNS that make serotonin originate in raphe nuclei which are in the middle of the brainstem. The most extensive and most intricate efferent system in the human brain is formed of these serotonin-producing neurons. While the more rostral raphe, the dorsal raphe nucleus, and the medial raphe nucleus innervate a large portion of the rest of CNS via diffuse projections, the most caudal raphe innervates the spinal cord. Serotonin regulates almost all behaviors and many other brain functions, and virtually every cell in the brain is close to a serotonergic fiber [5]. This neuromodulator is derived from the essential amino acid L-tryptophan, which is formed by the action of tryptophan hydroxylase [6]. In particular, serotonin is synthesized from the 5-hydroxytryptophan (5-HTP) catalysis by either tryptophan hydroxylase-1 (TPH1) (an enzyme produced in various tissues) or tryptophan hydroxylase-2 (TPH2) (an enzyme produced explicitly in the brain) [7].

The availability of serotonin in the synaptic cleft following its release is tightly regulated by the presence of serotonin transporter (SERT) in specific brain regions and functions. In brain regions where SERT is present, serotonin is quickly cleared from the synapse, leading to a more transient signal. Meanwhile, serotonin may linger longer in the synaptic cleft in specific brain regions lacking SERT. This condition potentially prolongs the serotonin effects on postsynaptic receptors. SERT is densely available in the ventromedial and dorsomedial regions of the dorsal raphe nucleus, dorsal to medial longitudinal fasciculus. Apart from that, it is also available in the hypothalamus, thalamus (depending on the nucleus), amygdala, putamen, caudate, hippocampus, insular cortex, prefrontal cortex, white matter, and cerebellar cortex (except vermis) [8]. The serotonergic neurons in these brain regions transmit their projections to different brain regions that involve various aspects such as mood, cognition, and autonomic functions.

Serotonin is transported and stored from the cytoplasm to synaptic vesicles via a Ca^{2+}-dependent mechanism. Following the release during the depolarisation state, serotonin is reuptake from the synapse by the SERT [9]. Meanwhile, the other brain regions with less or absence of SERT may have alternative mechanisms to modulate serotonin availability and possibly lead to a more extended presence of serotonin in the synapse. Serotonin mediates its role via seven different 5-HT receptors (i.e. 5-HT_1R to 5-HT_7R) which consist of 14 distinct subtypes (Table 1). The majority of these 5-HT receptors are G-protein coupled receptors (GPCRs) except the 5-HT_3 receptor (5-HT_3R), which is a ligand-gated cation channel [9]. The serotonergic GPCRs are formed from a standard structure and consist of seven transmembrane α-helices, coupled by three extracellular and three intracellular loops [10]. Upon the ligand binding, this intracellular loop and C-terminal tail interact with specific G protein families consisting of $G_{\alpha s}$, $G_{\alpha i/o}$, $G_{\alpha q/11}$, and followed by the production of second messengers [10]. Although the number of serotoninergic neurons is relatively small (i.e. 350 000 in humans), this neurotransmitter is essential in multiple physiological roles, including locomotion, eating, cardiovascular functions, cognition, memory, thermoregulation, reproduction, sleep, reward, mood, stress, and aggressiveness. Due to its significance in various physiological functions, the dysregulation of 5-HT receptors has been implicated in diverse pathogenesis of diseases, including but not limited to Alzheimer's disease, anxiety, schizophrenia, Parkinson's disease, depression, and sleep disorders [9, 11]. In the pain pathway, serotonin is released from the descending serotonergic path to the spinal cord dorsal horn as the means of the endogenous pain suppression system. Once produced, serotonin may bind to its receptors, either facilitating or inhibiting nociceptive transmission, depending on the nature of its serotonergic receptors.

Table 1 Types of serotonergic (5-HT) receptors.

Classification	Type of 5-HT receptor	Subtypes	Receptor activation effects in nociception	Main signal activation pathway	References
GPCR	5-HT_1	5-HT_{1A}	anti-nociceptive	$G_{\alpha i/o}$ protein	[12-14]
		5-HT_{1B}	anti-nociceptive		[13, 14]
		5-HT_{1D}	anti-nociceptive		[14]
		5-HT_{1F}	anti-nociceptive		[15]
GPCR	5-HT_2	5-HT_{2A}	pro-nociceptive	$G_{\alpha q/11}$ proteins	[13, 16]
		5-HT_{2B}	pro-nociceptive		[13, 16]
		5-HT_{2C}	pro-nociceptive		[13, 16]

LGIC	5-HT_3	5-HT_{3A}	pro-nociceptive	Cation channel	[16, 17]
		5-HT_{3B}	pro-nociceptive		[16, 17]
		5-HT_{3C}	pro-nociceptive		[16, 17]
GPCR	5-HT_4	-	pro-nociception		[18, 19]
GPCR	5-HT_5	5-HT_{5A}	anti-nociceptive	$G_{\alpha s}$ proteins	[14]
		5-HT_{5B}	unknown		[20]
GPCR	5-HT_6	-	pro-nociceptive	$G_{\alpha s}$ proteins	[18, 21]
GPCR	5-HT_7	-	pro-nociceptive	$G_{\alpha s}$ proteins	[22]
			anti-nociceptive		[14]

Abbreviation: GPCR – G protein-coupled receptor; LGIC – ligand-gated cation channel.

Amongst the 5-HT receptors, 5-hydroxytryptamine-6 receptor (5-HT_6R) stands out as a focal point of considerable scientific investigation. This glycoprotein receptor comprises 440 and 438 amino acids in humans, mice, and rats, respectively [7]. The immunohistochemical staining from previous studies has demonstrated the significant localization of 5-HT_6R on neuronal cell bodies, dendrites, and postsynaptic sites and its abundant expression in GABAergic, glutamatergic, and cholinergic neurons [23, 24]. 5-HT_6R is a particularly intriguing receptor subtype due to its exclusive distribution in the CNS. It possesses a comparatively low level of sequence homology (<50%) when compared to other types of serotonergic receptors [25]. Therefore, this review aimed to uncover the emerging role of 5-HT_6R in chronic pain research highlighting its function in pain modulation, neuronal excitability, and pain processing as reported in the pre-clinical studies. Additionally, the review may also discuss the reported therapeutic effects of potential compounds targeting this receptor, aiming to inform future pain management strategies and provide insights into the contribution of 5-HT_6R in chronic pain conditions.

2. 5-Hydroxytryptamine-6 Receptor

The 5-HT_6R was discovered in 1993 when this receptor was initially amplified and characterized from the rat striatum [26], and in the same year, the human 5-HT_6R was then cloned, characterized, and identified for chromosomal localization [27]. It belongs to a 7-transmembrane receptor that is connected to G_s protein [28]. 5-HT_6R possesses more extended and more flexible intracellular loop 3 structure which causing the expression yield of this receptor after the serotonin binding to be relatively low and unstable [25].

The 5-HT_6R is highly localized in brain areas, including olfactory tubercle, frontal and entorhinal cortices, dorsal hippocampus (i.e., dentate gyrus, CA1, CA2, and CA3 regions), striatum, and nucleus accumbens [9, 29], remarking its central expression centrally rather than peripherally. Lower expression of 5-HT_6R is reported in the hypothalamus, substantia nigra, amygdala, and various diencephalic nuclei [29]. In the hippocampus, 5-HT_6R is predominantly expressed on excitatory pyramidal cells and inhibitory GABA interneurons, although to a lesser extent [30]. In the spinal cord dorsal horn, the expression of this serotoninergic receptor subtype is found in excitatory interneurons receiving non-painful mechanical signals, particularly in the region below lamina II inner to lamina IV outer; the zone, which is responsible for receiving low-threshold mechanoreceptor signals [31, 32]. In specific, 5-HT_6R is mainly localised in the primary cilium of spinal cord dorsal horn neurons where this receptor subtype is believed to finely regulate the length

and signaling of cilia besides regulating the dendrite outgrowth and neuronal structure [32, 33]. In the animals, 5-HT_6R was reported to be expressed in dorsal root ganglion (DRG) neurons (peripherally) and glial cells (centrally) [19]. However, the peripheral expression of this receptor is relatively low in rodents [34]. These discoveries imply that the 5-HT_6R agonists or antagonists may implicate the GABAergic neurons to modulate the glutamatergic and/or cholinergic systems during the development of certain neurological disorders. For instance, the administration of a 5-HT_6R antagonist may attenuate the release of GABA if the GABAergic neuron is implicated.

Accumulating pieces of evidence have discovered the significant involvement of 5-HT_6R in the regulation of cognition, memory and learning, mood, and behavior [9]. Due to its abundance and essential roles, numerous research has targeted 5-HT_6R in neurodegenerative diseases marked by cognitive or memory deficits, including Alzheimer's disease, and psychotic and affective disorders, including schizophrenia [35, 36]. This links the 5-HT_6R to activating various neurotransmitter systems including glutamatergic, cholinergic, and dopaminergic pathways. Understanding its underlying mechanisms may offer insights into its modulation and possible effective cure in the future.

3. 5-HT_6R Signaling Mechanism

The 5-HT_6R mainly regulates the development processes of a neuron including its migration and differentiation [11]. This indicates the essential activation of 5-HT_6R to maintain physiological regulation of the CNS. Previous pre-clinical investigations discovered that 5-HT_6R could be spontaneously active without the presence of serotonin, although this receptor activation is frequently associated with endogenous serotonin binding [28]. This spontaneously activated state is termed as a constitutive activity of a receptor. 5-HT_6R is believed to trigger the intracellular signaling pathways on its own, although without the presence of a ligand. This exclusive auto-activation of 5-HT_6R facilitates the research to target and modulate it. The activation of this receptor involves several canonical (classical) and non-canonical (non-classical) pathways activation which are further discussed in this section.

3.1 Constitutive Activity of 5-HT_6R

Although the majority of therapeutic research on GPCRs focuses on the activation of receptors by endogenous agonists, the agonist-independent intrinsic constitutive activity has significant therapeutic potential and can be feasible in a variety of physiological and pathological contexts. Like other 5-HT receptors, 5-HT_6R possesses crucial elements known as ligand-independent constitutive activity where it can be highly active (i.e. able to couple to second messengers and elicit a signal) although with the absence of an agonist [28, 37]. The constitutive activity in wild-type receptors is critical for drug action and receptor modulation. The occurrence of several diseases, such as hyperfunctioning thyroid adenomas, familial male precocious puberty, retinitis pigmentosa, and metaphyseal chondrodysplasia, has been linked to naturally occurring mutations that result in constitutive receptor activation [37]. In this context, the constitutive activity of 5-HT_6R is essential for neuronal development, self-renewal of human neural stem cells, and neocortical radial migration [25]. Attenuating the constitutive activity of 5-HT_6R has been associated with a therapeutic effect on cognitive/memory deficits, as found in various neuropsychiatric disorders [35, 36].

Based on the findings in neuropathic pain rats, Mokhtar and colleagues [38] believed that the pathophysiology of diabetic neuropathic pain with cognitive comorbidities could be closely associated with the constitutive activity of 5-HT_6R in the spinal cord dorsal horn via dependent-activation of mTOR signaling. During the pathological excess of serotonin with the increased 5-HT_6R expression at the plasma membrane, the 5-HT_6R was observed to bind to cyclin-dependent kinase 5 (Cdk5) constitutively, activating the agonist-independent mechanism, which implicates the positioning and migration of pyramidal neurons during mouse corticogenesis [39]. Apart from Cdk5, the constitutive activity of 5-HT_6R can also be triggered by G protein-coupled receptor-interacting proteins (GIPs) like neurofibromin via 5-HT_6R-protein direct interactions and complex actions toward various signaling mediators that promote the neurite growth [28, 40]. In-vitro studies using JEG-2 and COS-7 cells have shown that native mouse 5-HT_6 receptors exhibit vigorous constitutive activity, even when expressed at very low levels as measured by cyclic adenosine monophosphate (cAMP) accumulation [37]. Similarly, another study reported vigorous constitutive activity of wild-type human 5-HT_6 receptors in cAMP signaling when expressed in HEK-293 cells, a system known for higher 5-HT_6 receptor expression compared to COS-7 cells [28, 41]. In contrast, human 5-HT_6 receptors expressed in COS-7 or CHO-K1 cells did not exhibit constitutive activity [42, 43]. Further exploration via site-directed mutagenesis has revealed that the constitutive activity of 5-HT_6R is related to the C-terminal region of the third intracellular loop of this receptor [37]. The activation of 5-HT_6R either via its high constitutive activity or the presence of agonist may further implicate canonical (classical) and/or non-canonical (non-classical) mechanisms involving the activation of several essential signaling pathways.

3.2 Canonical Pathway Activation of 5-HT_6R

The activation of the canonical pathway involves the coupling of $G_{\alpha s}$-protein, a subtype of heterotrimeric G proteins, following the 5-HT_6R activation. The binding of serotonin to the 5-HT_6R induces conformational changes activating the $G_{\alpha s}$ protein [9]. However, 5-HT_6R may also be coupled with $G_{i/o}$ protein, suggesting producing an inhibitory effect on pain [44]. In turn, this mechanism further results in the stimulation of adenylyl cyclase (AC) activity, an enzyme responsible for converting ATP into cyclic AMP (cAMP) [9]. The increased level of cAMP serves as a second messenger that activates protein kinase A (PKA) and consequently regulates several downstream signaling molecules, including CREB. These proteins then enter the nucleus and activate transcription factors including CREB and Elk1 through phosphorylation [10]. These mechanisms result in neuronal depolarisation that is essential for regulating cognitive functions including learning and memory, as well as processing and modulation of pain perception.

3.3 Non-Canonical Pathway Activation of 5-HT_6R

Compared to other subtypes of serotonergic receptors, there was limited information regarding the non-canonical pathway activation following the 5-HT_6R activation. However, the constitutive activity of 5-HT_6R may signal non-canonical cascades which engage in activating other cellular signaling cascades including extracellular signal-regulated kinase (ERK) ½, mitogen-activated protein kinases (MAPKs), Jun activation-domain-binding protein-1 (Jab-1), and light chain 1 subunit of the microtubule-associated protein-1B, and neuro-oncological ventral antigen 1 (Nova-1) [1, 9, 11].

Following the activation of 5-HT_6R, the activated PKA promotes the phosphorylation of ERK by a mechanism involving direct interplay of Fyn (Src family of non-receptor tyrosine kinases) with carboxyl terminus (C-terminal) of the 5-HT_6R [7, 11, 45] through a Ras- and mitogen-activated protein kinase activation-dependent pathway; a partial PKA-dependent and Rap1-independent signaling cascades. Fyn increases the activity of 5-HT_6R by elevating the receptor's surface expression without altering its total cellular protein expression. Furthermore, the activated 5-HT_6R also increases the phosphorylation of Fyn at Tyr-420 and induces ERK-1 and ERK-2 via Fyn- and PKA-dependent cascades [7]. The ERKs are then translocated to the nucleus, where they regulate gene expression, neuronal cell growth, differentiation, and survival. Apart from these essential roles, the activation of MAPK pathway is also demonstrated in the neurodegenerative processes including pathological pain, memory dysfunction and emotional disturbances [46, 47].

Besides that, 5-HT_6R also communicates directly with Jab-1 which causes the translocation of this protein from the neuronal cytoplasm to the nucleus. In the nucleus, Jab-1 interacts with c-Jun, a component of transcription factor Activator Protein-1 (AP-1), and promotes the Jab-1/c-Jun interaction [7, 45]. This receptor interaction also stimulates c-Jun activation via the binding of c-Jun and Jun D to AP-1 sites to increase their transcription factor activity [11]. Physiologically, the activation of the Jab-1 pathway is associated with the regulation of several genes' expression essential for neuronal growth, differentiation, stress responses, and possibly neuroplasticity. Apart from that, 5-HT_6R is also believed to interact with the light chain 1 (LC1) subunit of MAP1B protein (MAP1B-LC1), a classical microtubule-related protein that is highly expressed in the brain to modulate cognitive dysfunction. In a functional analysis study, Kim and colleagues [48] discovered that MAP1B-LC1 regulates explicitly signal transduction pathways downstream of 5-HT_6R by increasing its surface expression and reducing its endocytosis, which potentially reverses the cognitive dysfunction in the brain. While 5-HT_6R interaction with SNX14 and Nova-1 promotes its endocytosis and degradation, the association of 5-HT_6R with Fyn, Jab-1, or Map1B-LC1 increases the 5-HT_6R's expression at the neuronal plasma membrane likely by preventing its internalization.

Meanwhile, Doly et al. [49] have demonstrated that the constitutive activity of 5-HT_6R activates mammalian target of rapamycin (mTOR) protein pathway in the spinal cord neurons of neuropathic pain and associated cognitive malfunctioned models. As the C-terminal sequence of the 5-HT_6R was shown to be interacting with the mTOR protein, this finding possibly aids in generating a peptide capable of inhibiting the physical interaction of the receptor with mTOR [49]. mTORC1, a component of mTOR, is recruited to be activated via a dual mechanism involving classical (canonical) Pi3K/Akt/Tsc1,2/Rheb pathway and a direct interaction between the C-terminal domain of 5-HT_6R and mTOR [11]. However, this additional signaling pathway is not always associated with the constitutive activity of 5-HT_6R [28]. Doly and co-workers [49] have demonstrated that the inhibition of mTOR signaling via intrathecal administration of PZ-1388, a 5-HT_6R antagonist, has resulted in reduced tactile allodynia development and cognitive deficits. The anti-nociceptive action of 5-HT_6R was believed to result from the attenuation of the receptor's physical interaction with mTOR. A similar postulation was also agreed by Meffre and colleagues [50], where the mTOR protein activation by the 5-HT_6R could occur through the stimulation of PI3K/Akt/Rheb pathway as demonstrated in their *in-vitro* study using HEK-293 cells. In this study, the researchers have shown that the 5-HT_6R-induced mTOR activation was dependent on the canonical class I phosphatidyl inositol 3-kinase (PI3K)/Akt signaling proteins. The phosphorylation of tuberin (TSC2) by Akt attenuates the GAP activity of the tuberin/hamartin (TSC2/TSC1) complex, resulting in the elevated

levels of Ras homolog enriched in brain/GTP (Rheb-GTP) complex that in turn induce the mTOR activation [50]. The mTOR pathway via the PI3K/Akt activation is essential not only in neuroprotection, and neurodevelopmental processes (i.e. neuronal cell proliferation, growth, and synaptogenesis of dendrites and axons), but also in the nociceptive transmission following neuropathic pain and peripheral nerve injury [50, 51].

Besides the mTOR signaling pathway, 5-HT$_6$R can also interact with other intracellular signaling pathways that do not involve the receptor's constitutive activity, including cyclin-dependent-kinase5 (Cdk5) and RhoA-dependent pathways [9, 38]. The activation of the non-canonical Gαq/11-RhoA pathway by 5-HT$_6$R modulates nuclear actin and elevates histone acetylation and chromatin accessibility in the neurons [52]. In terms of ionic regulation, it has been shown that the 5-HT$_6$R activation also inhibited G protein-coupled inwardly rectifying potassium channel (GIRK) conductance in the neurons as shown in the rat's striatal cholinergic interneurons by Bonsi and colleagues [53]. The activated PKA following the 5-HT$_6$R activation also phosphorylates specific serine or threonine residues on potassium (K^+) channels (e.g., voltage-gated K^+ channel) or causes K^+ currents inactivation that results in persistent neuronal depolarisation [54]. The connection to these multiple signaling pathways may explain the relationship between 5-HT$_6$R and the cholinergic, glutamatergic, and dopaminergic neuronal submission in the CNS [55]. The overall postulated signaling mechanisms following 5-HT$_6$R activation are illustrated in Figure 1.

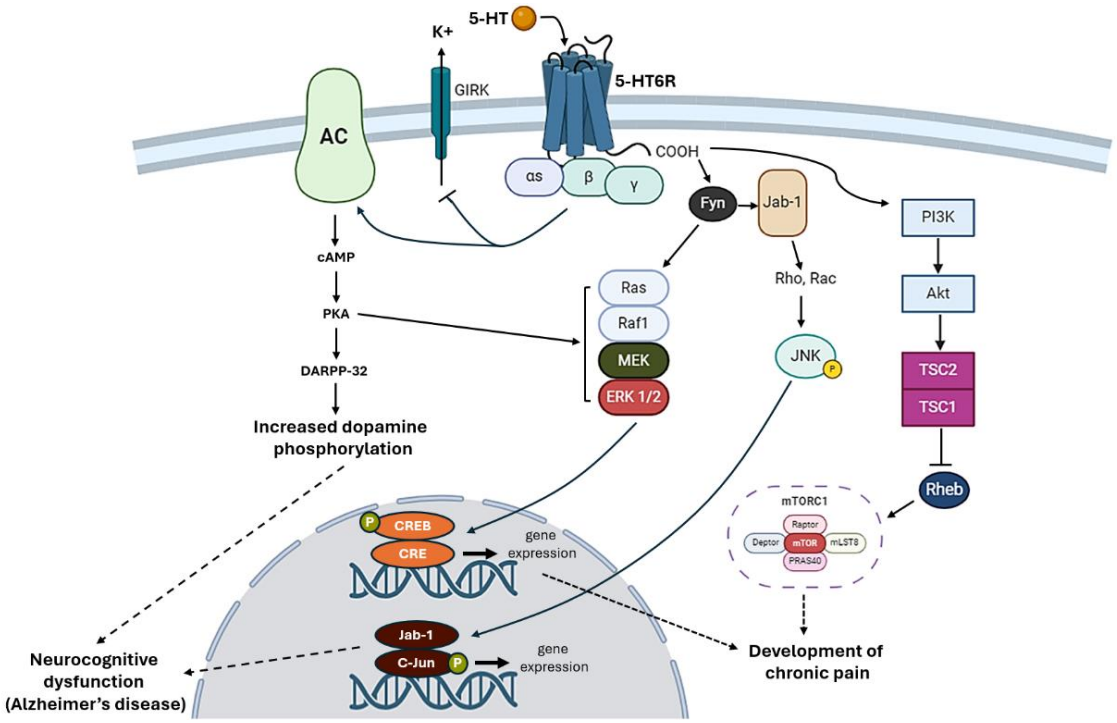

Figure 1 Postulated mechanisms involving canonical and non-canonical pathways following the activation of 5-HT$_6$R. The activation of 5-HT$_6$R via canonical path consists of the coupling of Gas protein, resulting in the activation of AC/cAMP/PKA that stimulates the transcription of CREB and Elk1 and neuronal depolarization. This pathway activation is associated with cognitive function regulation as well as pain processing and modulation. Meanwhile, the activation of the non-canonical pathway following 5-HT$_6$R activation involves the activation of other cellular signaling cascades, including ERK ½, MAPKs, Jab-1, and Nova-1.

4. 5-HT$_6$R Receptor Action Is Region-Specific

The effects of 5-HT$_6$R activation or inhibition vary depending on the brain region in which they are expressed, thus highlighting their region-specific role in neurophysiological processes. The brain striatum, hippocampus, and cortex are among the main areas where this serotoninergic receptor is highly available and contributes differently to cognitive and sensory functions. In the striatum, 5-HT$_6$R plays critical functions in stimulus-response learning and motor control, as demonstrated in studies where their overexpression in the dorsomedial striatum disrupts instrumental learning. However, this effect is not observed when 5-HT$_6$R is increased in the dorsoventral striatum, which suggests that even with the same brain structure, the functional effects of 5-HT6 receptor modulation can differ significantly [56].

In the hippocampus, which is crucial for spatial and episodic memory, the application of 5-HT$_6$R antagonists has been shown to enhance learning and memory, likely by modulating cholinergic and glutamatergic neurotransmission. Dawson et al. [57] demonstrated that the antagonism of 5-HT$_6$R selectively enhances excitatory neurotransmission through glutamate release in the hippocampus of freely moving rats without altering basal levels of other neurotransmitters. Unlike in striatum where excessive 5-HT$_6$R receptor activation disrupts new learning, hippocampal-dependent tasks like Morris water maze remain largely unaffected by the overexpression of 5-HT$_6$R. This contradiction indicates that 5-HT$_6$R in the hippocampus does not strongly interfere with memory acquisition but possibly modulates the synaptic plasticity and consolidation processes. In Alzheimer's disease and schizophrenia studies, it has been shown that the pharmacological manipulation of 5-HT$_6$R using its antagonists (i.e. EMD 385088 and SB-399885) modulates the field inhibitory postsynaptic potentials of the dorsal hippocampus mediated by γ-butyric amino acidergic (GABAergic) interneurons on pyramidal cells, suggesting that 5-HT$_6$R inhibition may involves GABAergic transmission modulation for its anti-amnesic effects [58].

Meanwhile, 5-HT$_6$R influences executive functions, including attention and decision-making, upon its activation within the brain's prefrontal cortex. The antagonism of 5-HT$_6$R has been linked to increased extracellular levels of monoamines, which potentially enhance cognitive flexibility and working memory. This positive effect of 5-HT$_6$R attracts researchers to develop treatments for neuropsychiatric disorders such as Alzheimer's disease and schizophrenia [57, 59]. The region-specific action of 5-HT$_6$R underscores their diverse functional roles across different neural circuits that make them a promising yet complex target for therapeutic interventions. Understanding how 5-HT$_6$R contributes to various cognitive and behavioral processes across different brain regions makes it essential in refining pharmacological strategies targeting neurological and psychiatric disorders.

5. Involvement of 5-HT$_6$R in the Development and Maintenance of Chronic Pain

The researchers have shifted their focus on 5-HT$_6$R as a potential target to treat chronic pain particularly neuropathic pain. Although the role of 5-HT$_6$R is well-documented in the brain areas involving cognition, its expression is also detected in excitatory interneurons of the spinal cord dorsal horn that is involved in tactile perception [1]. The earlier findings discovered its prominent protein distribution and/or mRNA expression in the cortex, thalamus, amygdala, periaqueductal gray, spinal cord, and dorsal root ganglia, defining its potential role in analgesia and pain modulation [10]. Although 5-HT$_6$R is one of the most recently discovered among the 5-HT family, this receptor

is found to be expressed in the excitatory interneurons of the spinal cord dorsal horn [31, 44], signifying its prominent contribution to pain transmission and development.

Local peripheral post-treatment with the selective 5-HT_6R antagonist, SB-258585, has resulted in the significant attenuation of secondary mechanical allodynia and hyperalgesia in the formalin-evoked nociceptive rat [34]. Although the development of these pain responses is possibly derived from the simultaneous activation of several 5-HT receptors following the binding of serotonin, the peripheral activation of 5-HT_6R may induce prolonged occurrences of these neuropathic-like symptoms. Extending their research using the same animal model to investigate the involvement of spinal 5-HT_6R, Godínez-Chaparro et al. [19] have found that the intrathecal treatment of 5-HT_6R agonist EMD-386088 strongly increased the pain responses in both hind paws while the subsequent intrathecal injection of 5-HT_6R antagonist (SB-258585) markedly inhibited these pain responses. Based on these findings, the researchers postulated that the spinal serotonin released from the serotoninergic projections possibly activates pre- or post-synaptic 5-HT_6R at the DRG or spinal cord and consequently facilitates the development and maintenance of secondary allodynia and hyperalgesia. These increased pain responses could be attributed to the direct activation of 5-HT_6R localized on the DRG neurons or central terminals of the primary afferent fibers in the spinal cord dorsal horn, direct stimulation of 5-HT_6R in excitatory interneurons and projection neurons, and direct activation of 5-HT_6R expressed on the glial cells (i.e. microglia or astrocytes) [19].

Similar findings were also demonstrated by Martin and colleagues [32] where the inverse agonist activity of SB-258585 at 5-HT_6R is responsible for the attenuation of pain activities. The anti-allodynic effect of SB-258585 was then inhibited by intrathecal administration of a neutral antagonist, further indicating that the 5-HT_6R constitutive activity, rather than tonic activation of spinal 5-HT_6R elicited by serotonin, is responsible for spinal nerve ligation-induced allodynia. In conjunction with these findings, the anti-allodynic action brought on by the intrathecal injection of SB-258585 highlights the critical role of constitutively active 5-HT_6R in the spinal cord region that contributes to the maintenance of neuropathic pain. The researchers further discovered that 5-HT_6R constitutively activates the non-canonical mTOR signaling pathway through the assessment of mTOR phosphorylation (at Ser^{2448}) and its downstream substrate S6 (at $Ser^{240/244}$), which may lead to tactile and thermal allodynia-induced by chemotherapy and traumatic nerve injury.

In another study of formalin-induced nociceptive rat model, Cervantes-Durán and co-workers [60] investigated the role of 5-HT_6R in fluoxetine-induced nociception and anti-nociception condition. The researchers discovered that fluoxetine (selective serotonin reuptake inhibitors, SSRIs) has augmented pain response (i.e. thermal hyperalgesia) at the peripheral ipsilateral paw possibly by activating the peripheral 5-HT_6R since its pro-nociceptive effect was significantly suppressed by 5-HT_6R antagonist, SB-258585. It is postulated that fluoxetine perhaps promotes serotonin release and prevents its reuptake, which in turn causes the serotonin to stimulate peripheral 5-HT_6R leading to intense pain response in the mice. Next, the researchers administered fluoxetine intrathecally and discovered that the spinal anti-nociceptive effect of fluoxetine was not associated with 5-HT_6R, but other serotoninergic receptors (i.e., 5-HT_{1A}, 5-HT_{1B}, 5-HT_{1D}, and 5-HT_{5A}). Although the role of 5-HT_6R in pain processing at the peripheral and central levels is not well understood, this study brings the insight that 5-HT_6R may be involved in peripheral pro-nociception but not in spinal anti-nociceptive effects following the fluoxetine administration. However, it is essential to consider the types of pain responses and the projection pathway involved. Different subtypes of allodynia and hyperalgesia are developed and maintained by peripheral and central changes in which each of

these pain responses innervate their nociceptive pathways. Moreover, the etiology of allodynia and hyperalgesia produced following nerve injury-induced neuropathic pain could differ from diabetic neuropathic pain [61]. Meanwhile, Mokhtar et al. [38] discovered that the constitutive activity of 5-HT_6R rather than its activation by endogenous serotonin binding, plays key role in the development of diabetic neuropathic pain via dependent activation of mTOR as the administration of 5-HT_6R inverse agonists have improved mechanical hypersensitivity and co-morbid cognitive deficits following diabetes. The inhibition of 5-HT_6R following the intrathecal administration of the antagonist SB-258585 suggests that the spinally located 5-HT_6R contributed to this pathomechanism as its expression in the excitatory interneurons receiving nociceptive inputs from low threshold mechanoreceptors were previously reported [31].

It is also possible that the 5-HT_6R activation could influence the synaptic activity and glutamatergic transmission involving N-methyl-D-aspartate receptors (NMDARs) during the transmission of nociceptive signals. In mice hippocampal slices, the attenuation of 5-HT_6R activity by SB-271046 has resulted in the elevation of synaptic-glutamate-associated synaptic potentials mediated by NMDARs and non-NMDARs without affecting the glutamate release. These findings elaborate that the 5-HT_6R influences post-synaptic mechanisms in the neural network to regulate glutamate transmission rather than altering the synaptic glutamate release [30]. Similarly, Woods and colleagues [62] have revealed that the activation of 5-HT_6R via the use of the 5-HT_6R agonists E-6801 and EMD 386088 in the memory deficit rats has resulted in the strong reversal of hippocampal cholinergic and glutamatergic mechanisms associated with memory and learning function. Although no studies have yet investigated the role of 5-HT_6R in NMDAR activation-induced chronic pain development, 5-HT_6R may be involved in mechanisms that lead to the activation of specific NMDAR subtypes during chronic pain pathogenesis. This is supported by well-established evidence that NMDAR activation, particularly the NR2B subtype, plays a key role in the development of chronic neuropathic pain and neurotoxicity [63, 64]. The previous findings of 5-HT_6R in chronic pain investigation are summarised in Table 2.

Table 2 Reported involvement of 5-HT_6R in pre-clinical investigations including chronic pain models.

In-vivo or in-vitro models	Findings	References
Electrophysiological study on hippocampal slices of C57BL/6 mice *(in-vitro)*	5-HT_6R affects post-synaptic mechanisms in the neural network to regulate glutamate transmission rather than modifying the synaptic glutamate release.	[30]
Chloroquine-induced pruritus (itch) mice *(in-vivo)*	5-HT_6R is believed to be involved in sensory itch processing. However, the use of 5-HT_6R agonist or reversal by 5-HT_6R antagonist did not change the histamine-induced pruritus in mice.	[65]
Streptozotocin-induced painful diabetic neuropathy *(in-vivo)*	Constitutive activity of 5-HT_6R and mTOR via the action of rapamycin (5-HT_6R inverse agonist) is involved in the development of diabetic neuropathic pain (tactile hypersensitivity and mechanical hyperalgesia) with impaired cognitive function	[38]
Diabetic Balb/c mice	Systemic 5-HT_6R activation contributes to the neuropathic pain symptoms (thermal hyperalgesia) during the pathophysiology of diabetic neuropathy	[61]
Spinal nerve ligation-induced neuropathic pain *(in-vivo)*	No significant changes in the 5-HT_6R expression in spinal cord dorsal horn of the rat following nerve injury Spinal 5-HT_6R plays a role in the nociceptive processing (maintainance) of neuropathic pain	[18]
Spared sciatic nerve injury rat model *(in-vivo)*	Microinjection of 5-HT_6R antagonist SB-258585 into ventrolateral orbital cortex significantly antagonised serotonin-induced allodynia inhibition in the rat, remarking anti-nociceptive role of 5-HT_6R in descending pain inhibitory circuits.	[66]
Formalin-induced acute nociceptive rat model *(in-vivo)*	The development of formalin-induced long-term secondary allodynia and hyperalgesia relies on the presence of an excitatory descending serotonergic pathway involving 5-HT_6R activation	[19]

6. Pain Inhibitory Effect of 5-HT_6R Activation

Although the previous studies reported the significant contribution of 5-HT_6R as pro-nociceptive, however, it is also associated with an anti-nociceptive effect [44]. 5-HT_6R may exert either a pro- or anti-nociceptive impact depending on the region of the organ or system in which it is activated. This condition is closely related to the release of serotonin in the area as the role of serotonin in pain processing depends on the type of nociceptive stimuli and the nature of serotoninergic receptors it binds to [19]. For instance, since 5-HT_6R is also found to be densely expressed in the inhibitory interneurons, the activation of this receptor may result in eliminating pain signals at the synapse. It is also agreed by Miyahara and colleagues [65] that the serotonergic axons terminating on the inhibitory neurons expressing 5-HT_6R may produce inhibition of sensory itch processing in the CNS.

Apart from the ascending pain pathway, the serotoninergic system is also available in descending pain facilitatory and inhibitory circuits. A stable baseline of pain perception and processing may be attributed to the normal regulation of this pain circuit. Unfortunately, any abnormality in this system can contribute to either analgesia or hyperalgesia [67]. As 5-HT_6R is also densely found in the descending pain inhibitory system, its constitutive activity may lead to suppression of pain transmission. The descending pain system contains central regions that modulate pain transmission, including the anterior cingulate cortex (ACC), rostroventromedial medulla (RVM), and dorsal reticular nucleus of the medulla (DRN). In contrast, the descending inhibitory serotoninergic system derives from periaqueductal gray (PAG), RVM, and caudal ventrolateral medulla (VLM) [6]. In the physiological state, the descending inhibition derived from the RVM attenuates the pain transmission via serotoninergic neuron activation, which further leads to numerous neurotransmitters' release in the primary afferent neuron along the spinal projection neuron and inhibitory interneuron [68].

Regarding 5-HT_6R, the anti-nociceptive effect of this receptor has been reported in the descending facilitation system. In spared nerve injury rat, the microinjection of selective 5-HT_6R antagonist SB-258585 into ventrolateral orbital cortex, a region involving descending pain inhibitory system, has resulted in the antagonism of the serotonin-induced inhibition of allodynia. This study proposed that the serotonergic receptors, including 5-HT_6R, are possibly involved in mediating the depression of serotonin-induced allodynia in the VLO, although these serotonergic receptors do not exert prolonged modulatory action on allodynia development [66]. In the brainstem, RVM projects the pain impulses to the spinal cord via the dorsolateral funiculus, which in turn releases various neuromodulators, including dynorphin, cholecystokinin, calcitonin gene-related peptide (CGRP), and serotonin, to facilitate the development and maintenance of chronic pain. During the pathological condition, the activation of ON cells of RVM by cholecystokinin is speculated to contribute to the behavioral hyperalgesia and allodynia responses. It is well-known that serotonin may either facilitate and/or inhibit nociceptive transmission depending on the nature of its serotonergic receptors. However, during the pathological condition involving long-term nociception, this descending pain system involving the serotonergic system could be altered from exerting an anti-nociceptive effect to a pro-nociceptive effect. This is agreed by Leong and colleagues [69] that the switched role of serotonergic system activation was shown in their spinal nerve ligation model which it is speculated to be caused by the significant serotonergic neuronal cell death (apoptosis) in the spinal cord region. In the development of the acute nociceptive model, serotonergic descending

inhibition appears to be crucial for the development of primary hyperalgesia in formalin-evoked pain responses. In contrast, the descending facilitation is believed to contribute to the subsequent occurrence of hyperalgesia [19].

At the supraspinal level, the activation of 5-HT_6R is postulated to reduce the pain transmission in the post-ictal anti-nociception elicited by pentylenetetrazole-induced tonic-clonic seizures. In this rat model, Freitas and colleagues [70] have explored the involvement of several serotoninergic receptors including 5-HT_6R via the peripheral, intraperitoneal administration of non-selective serotonin receptors antagonist, methiotepin. It was found that the lower doses of this drug (1 and 0.5 mg/kg) effectively reduced the post-ictal anti-nociception during the seizures. Interestingly, the administration of methiotepin at moderate and higher doses (2 and 3 mg/kg) has caused an unexpected facilitatory effect on post-ictal anti-nociception in the later episodes of seizures (~90 min). The researchers have deduced that the inhibition of the specific serotoninergic receptors post-synaptically involving the dorsal raphe nucleus region possibly lowers the intensity of the post-ictal anti-nociception during tonic-clonic seizures.

7. 5-HT_6R Agonists and Antagonists Identified from Neurological Studies

Since the discoveries of the human 5-HT_6R [27], diverse novel and selective compounds have been developed by applying 5-HT_6R-specific high-throughput screening technologies. Several selective antagonists of this receptor with highly potent ligands have been successfully created. The highest selectivity and potency antagonists are SB-399885 (300-fold selectivity for 5-HT_6R than other 5-HTRs), Ro 04-6790, Ro 63-0563, SB-258585 (100-fold selectivity), and SB-271046 (50-fold selectivity) [7]. Unfortunately, some of these compounds possess a restricted capacity to cross the blood-brain barrier (BB) and are found to be frequently used orally, such as SB-271046 and Ro04-6790 [71, 72]. Meanwhile, other 5-HT_6R antagonists such as SB-699929, SB-357134, and SB-399885 seem to possess greater pharmacokinetics and pharmacological profiles compared to SB-271046 and SB-258585 [73]. Other reported 5-HT_6R antagonists include SAM-531, SGS-518, PRX-07034, BVT-74316, R-1485, SYN-114, SYN-120, AVN-322, AVN-211, SB-742457, and SUVN-502 especially in the investigations on cognitive disorders [7]. Meanwhile, the discoveries from pre-clinical research on animal models mimicking human-like neuropathic pain with a high translational value have demonstrated the potent analgesic effect of 5-HT_6R ligands, suggesting that these compounds could be novel therapeutic candidates for the treatment of chemotherapy- and trauma-induced peripheral neuropathy (CIPN) [1, 32]. Since the constitutive activity of 5-HT_6R either at canonical G_s signaling or non-canonical signaling, has been elaborated in various pathological states, the 5-HT_6R ligands can be developed as neutral antagonists, inverse agonists, or both at various 5-HT_6R-operated signaling activation to understand the cellular mechanisms governing the different 5-HT_6R signal transduction mechanisms and constitutive versus agonist-dependent receptor activation [74].

Although various 5-HT_6R antagonists and inverse antagonists have been developed, relatively fewer substances identified as 5-HT_6R agonists have been reported. To date, the agonists of 5-HT_6R as reported in the previous literature are WAY-466, WAY-208466, WAY-181187, EMD386088, E-6801, LY586713, and R-13c [73]. Amongst these agonists, R13-c, WAY-181187, and EMD386088 exhibited 50- and 20-fold selectivity for 5-HT_6R, respectively. Meanwhile, E-6801 and E-6837 display partial effects of 5-HT_6R agonism [75]. WAY-181187 and ST1936 were reported to promote 5-HT_6R activation by increasing the phosphorylation of Fyn [76]. WAY-181187 and WAY-208466 were

reported to have high affinity binding to human 5-HT_6R. The acute subcutaneous treatment of WAY-181187 has significantly induced a robust increase in extracellular GABA levels without affecting the level of norepinephrine, dopamine, serotonin, or glutamate. However, this 5-HT_6R agonist did not affect the extracellular levels of GABA in the thalamus, an essential region for pain processing [29]. The previous discoveries on 5-HT_6R agonists and antagonists specifically in the nociceptive research are shown in Table 3.

Table 3 Potential agonists and antagonists of 5-HT_6R in nociception from pre-clinical investigations.

Treatment(s)	Dosage	Action on 5-HT_6R	Model	Treatment effects	References
SB-258585	0.00001-0.001 nmol/(intraplantar)	antagonist	Formalin-induced nociceptive rat	- attenuated secondary mechanical allodynia and hyperalgesia in formalin-evoked nociceptive rat	[18]
SB-258585	- 5 µmol/kg, i.p. (SB-258585) - 5 and 25 µmol/kg, i.p. (PZ-1388)	antagonist	Oxaliplatin-induced peripheral neuropathy rat	- attenuation of cold allodynia and mechanical hyperalgesia in SNL rat - involvement of constitutive activity rather than tonic activation of 5-HT_6R in the spinal cord region	[34]
SB-258585	*(i.p.)	antagonist	Spinal nerve L5 ligation rat and oxiplatin-induced neuropathic pain rat	reversal of tactile allodynia, thermal allodynia, and improved cognitive impairments (episodic memory and social recognition memory)	[32]
SB-258585	0.0002-0.02 nmol, Intracerebral	antagonist	Spared sciatic nerve injury rat	Microinjection of SB-258585 into VLO significantly antagonized serotonin-induced allodynic suppression	[49]
SB-258585	- 3, 10, 30 mg/kg (i.p.) - 0.01, 0.1 and 1 nmol (i.t)	antagonist	Diabetic neuropathy mice	Systemic but not spinal blockade of SB-258585 has attenuated thermal hyperalgesia and spinal5-HT_6R protein level in the mice	[66]
SB-258585, SB-399885	- 0.001-0.1 nmol/paw (SB-399885) - 0.01-1 nmol/paw (SB-258585)	Selective antagonists	Formalin-induced acute nociceptive rat	Both SB-399885 and SB-258585 exerted no effect on 1% formalin-induced flinching behavior, but have antagonized the spinal pro-nociceptive effect of EMD-386088 in the rat	[61]
SB-258585, WAY 208466	- SB-258585 (antagonist; 100 nmol, i.t.)	Antagonist (SB-258585) and agonist (WAY-208466)	Spinal nerve injury rat	- Suppression of tactile allodynia in a dose-dependent manner in L5-L6 spinal nerve injury rat by SB-258585.	[21]

	- WAY-208466 (agonist; 100-1000 nmol, i.t.)			- co-administration of WAY 208466 (5-HT_6R agonist) have prevented the anti-allodynic effect of SB-258258 (5-HT_6R antagonist)	
SB-258585, EMD-386088		Agonist (EMD-386088) and antagonist (SB-258585)	Formalin-induced acute nociceptive rat	Significant increase of secondary mechanical allodynia and hyperalgesia in rat's hind paws following administration of EMD-386088. The administration of SB-285585 subsequently inhibited these intense pain responses in both hind paws.	[4]
SB-742457	2 or 5 mL/kg (i.p.)	antagonist	Partial sciatic nerve ligation rat	- 5-HT_6R antagonist exerted pro-cognitive effects by improving learning and memory impairment in neuropathic pain conditions. - 5-HT_6R antagonist may lower the dosage of gabapentinoid and indirectly reduce the drug's side effects when being co-administered.	[19]
SB-271046	1,5, and 10 mg/kg (i.p.)	selective antagonist		- lowers pre-formalin distance locomotion, grooming, rearing, and defecation during formalin test, dose-dependently - increases plasma corticosterone in formalin-evoked pain in rat	[77]
PZ-1388, PZ-13866, PZ-1179	25 µmol/kg	Selective inverse agonist	Painful diabetic neuropathy rat	Exertion of anti-hyperalgesic effect; improved pain responses for a minimum of 180 min in the rat	[38]
Methiotepin	0.1 mg/kg	Non-selective 5-HT_1R, 5-HT_6R and 5-HT_7R antagonist	chronic constriction injury-induced neuropathic pain mice	Significant abolishment of cardamon in-induced anti-hyperalgesic and anti-allodynic effects after the methiopin's pre-treatment in the mice	[6]
Methiotepin	0.5, 1.0, 2.0 and 2.0 mg/kg (i.p.)	Non-selective 5-HT_1R, 5-HT_6R and 5-HT_7R antagonist	Tonic-clonic seizure-induced anti-nociception	Lower dose of peripherally-administered methiotepin (0.5 and 1.0 mg/kg) produced pro-nociceptive effect whereas moderate and moderate-	[70]

				to-high-dose of methiotepin (2.0 and 3.0 mg/kg) exerted anti-nociceptive effects during episodes of seizures in rat.	
WAY 208466, SB-399885	* (i.t.)	Highly-selective agonist (WAY 208466), antagonist (SB-399885)	Chronic constriction injury to infraorbital nerve (trigeminal neuropathic pain) rat	Administration of WAY 208466 significantly increased mechanical thresholds (anti-allodynia) while the treatment of SB-399885 did not change the mechanical threshold of the mice	[78]

**limited access to data.*

Abbreviation: i.p., intraperitoneal; i.t., intrathecal.

8. Possible Side Effects Profiles of 5-HT$_6$R Agonism or Antagonism

Since serotonin is diversely distributed throughout CNS and involves a variety of signaling pathways activation, it becomes a potential target for therapeutic interventions. Given the multifaceted roles of the serotonergic system, targeting the 5-HTRs with precise attributes and specific localization could chart a groundbreaking course in drug development. Since the 5-HT$_6$R was recently discovered, little is known about its side effects profile, as investigated in previous pre-clinical research. However, some researchers reported its therapeutic potential considering the drug tolerance and relevancy.

Although research on 5-HT$_6$R agonists and antagonists is ongoing, previous studies have reported their therapeutic potential. In a neuropathic pain rat with significant memory and learning deficits, oral administration of 5-HT$_6$R antagonist SB-742457, in combination with a gabapentinoid, effectively attenuated cognitive impairments while enhancing the analgesic effects of the gabapentinoid without causing significant motor side effects [4]. Similarly, SB-742457 has potential as a promising drug target for cognition improvement in Alzheimer's disease through the compelling evidence of 5-HT$_6$R in learning and memory processes demonstrated in various pre-clinical and clinical studies [78]. Notably, the strategy of linking SB-742457 to other pharmacophores has been widely explored to counteract cognitive deficits which highlights their potential as a multitarget approach in drug development. Additionally, this drug was developed as a 5-HT$_6$R antagonist and clinical trials have demonstrated that it has greater affinity and better CNS penetration for treating Alzheimer's disease- and schizophrenic-associated cognitive deficits [79, 80]. This study further supports the idea that combining 5-HT$_6$R antagonists with other analgesics may allow for lower drug dosages and reduced treatment frequency while minimizing adverse effects.

Meanwhile, SB-258585 has effectively attenuated the neuropathic pain symptoms via the inverse agonism of 5-HT$_6$R, specifically at G_s and Cdk5 signaling [74]. The effect of this inverse agonist compound is reported to be typically well-tolerated and undoubtedly more relevant than other compounds such as mTOR inhibitors which have significant side effects associated with their immunosuppressive action [81]. Moreover, since 5-HT$_6$R is predominantly localized in the CNS, it is highly possible that targeting 5-HT$_6$R by certain chemical compounds or ligands may have no peripheral adverse effects after the administration [82].

9. Conclusion

Based on the previous discoveries of 5-HT$_6$R, it can be deduced that this serotoninergic receptor subtype plays essential roles in various physiological mechanisms. Since the discovery of 5-HT$_6$R is still new and available data on its signaling mechanisms are still lacking [5], the majority of the studies have reported its powerful effect on cognitive and pain behavior responses. However, the deeper investigation involving the molecular and genetic mechanisms following its activation or inhibition is scarcely reported. Since this receptor showed significant involvement in the pathogenesis of neurological disorders particularly related to chronic pain, further investigations on its availability in other body regions either peripherally or centrally is essential for the future strategy of pain management. Based on the previous research, most of the studies have higher preference on SB-258585 in antagonising the 5-HT$_6$R antagonist in the effort of understanding the mechanism and targeting 5-HT$_6$R. Although the research on 5-HT$_6$R is still in its early stages, some

of the 5-HT_6R antagonists such as idalopirdine, SB-742457, and SUVN-502 have been investigated in clinical trials for Alzheimer's disease. Further research is required to establish the safety and efficacy of these potential drugs before they can be approved for the patient's use.

Abbreviations

AC	adenylyl cyclase
ACC	anterior cingulate cortex
Akt	protein kinase B
AP-1	transcription factor Activator Protein-1
ATP	adenosine triphosphate
cAMP	cyclic adenosine monophosphate
DRG	dorsal root ganglia
DRN	dorsal reticular nucleus of the medulla
cAMP	cyclic adenosine monophosphate
Cdk5	cyclin-dependent kinase 5
CGRP	calcitonin gene-related peptide
CNS	central nervous system
CRE	cAMP response element
CREB	cAMP response element-binding protein
c-Jun	c-Jun activation domain-binding protein-1
ERK	extracellular signal-regulated kinase
Fyn	Fyn kinase
GABA	γ-amino butyric acid
GIPs	G protein-coupled receptor-interacting proteins
GIRK	G-protein-coupled inwardly rectifying potassium channel
GPCRs	G-protein coupled receptors
Jab-1	Jun activation-domain-binding protein-1
JNK	c-Jun N-terminal kinases
LGIC	ligand-gated cation channel
MAPKs	mitogen-activated protein kinases
mTOR	mechanistic Target of Rapamycin
NMDAR	N-methyl-D-aspartate receptor
Nova-1	neuro-oncological ventral antigen 1
PI3K	phosphoinositide 3-kinase
PKA	protein kinase A
Ras/Raf1	serine/threonine kinase
Rheb	Ras homolog enriched in brain
RVM	rostroventromedial medulla
SERT	serotonin transporter
TPH1	tryptophan hydroxylase-1
TPH2	tryptophan hydroxylase-2
TSC2/TSC1	tuberin/hamartin complex
VLM	caudal ventrolateral medulla

5-HT	serotonin
5-HTP	5-hydroxytryptophan
5-HT_6R	5-hydroxytryptamine-$_6$ receptor

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