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Review

New tricks for an old slug: Descending serotonergic system in pain

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Abstract: A large body of research including animal and human studies has confirmed the crucial role of the serotonin (5-HT) system in the regulation of nociception and chronic pain-related behaviors. In recent years, the functional status of the 5-HT system in descending inhibition and facilitation of spinal nociceptive processing has been reevaluated by novel genetic manipulation techniques and selective agents for 5-HT receptor subtypes. Although these studies shed more light on several aspects of descending 5-HT and spinal 5-HT receptors functioning in descending modulation in pain perception, the current knowledge about the specific role of descending 5-HT system in the induction and maintenance of persistent pain remains fragmentary. In this paper, we review the available data from recent studies of the inhibitory or facilitatory influence from descending 5-HT-spinal 5-HT receptor system in acute and persistent pain, attempt to dissect the involvement of this signaling pathway in neural circuits of maintenance of persistent pain and discuss some issues that need to be considered for further pain research.

Key words: 5-HT; 5-HT receptors; descending modulation; spinal cord; pain

古曲新韵：下行性5-羟色胺系统在疼痛机制中的作用

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摘 要：大量临床和基础的研究提示5-羟色胺(5-HT)系统参与了生理痛觉的调节和慢性痛的机理。近来，随着全新的基因调控技术和药理上特异性5-HT受体亚型试剂的应用，人们对5-HT系统在脑干下行痛觉抑制和易化机理中的功能又有新的认识。尽管这些研究揭示了下行性5-HT传导及其脊髓内受体在痛觉调制的新功能，但5-HT系统在慢性痛的形成和维持等方面的作用仍了解甚少。本文回顾了近年来在此领域已取得的进展，力图更新我们对这一经典神经递质和相关受体信号系统及其可塑性变化在慢性痛机理中的认识，进而引导我们寻求新的战略来发展有效的临床抗慢性痛药。

关键词：5-羟色胺；5-羟色胺受体；下行性调控；脊髓；疼痛
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As one of the most ancient biogenic amine or signaling molecules, serotonin (5-hydroxytryptamine or 5-HT) is early found within gastrointestinal tract and blood platelets and has been implicated in a large variety of physiological or behavioral functions from autonomic activity to cardiovascular regulation [1]. Only later is it found in the central nervous system and influences neuronal activity as a neurotransmitter via distinct 5-HT receptors, involving in tremendously important brain functions including emotional behavior and perception of pain [2]. Although 5-HT-containing neurons are clustered in the exact midline of the brainstem termed the raphe nuclei and only occupy 1%–2% of the whole body 5-HT in mammalian brain, its ascending and descending fibers are vastly distributed throughout the brain and spinal cord, respectively. 5-HT's diverse effects are dependent on pharmacologically and functionally distinct 5-HT receptor subtypes classified to seven

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families termed 5-HT_1 through 5-HT_7, the cell types expressing these receptors, and the integration of 5-HT and its receptor interactions from the respective local circuitry. An extensive literature has shown that altered ascending 5-HT system in the cortical cortex plays a critical role in the pathogenesis of some psychiatric diseases, particularly in depression, anxiety and schizophrenia [3]. Most effective drugs such as antidepressants and antipsychotics are thought to influence the 5-HT signaling in the brain. Meanwhile, there have been numerous progresses shedding light on the function of descending 5-HT system in the modulation of spinal nociceptive processing and behavioral hypersensitivity after tissue and nerve injury. This review focuses on recent evidence in research with the rapid advances in modern molecular, genomic and pharmacological techniques for the favorable involvement of descending 5-HT and spinal dorsal horn 5-HT receptor subtypes in the maintenance of persistent pain in animal models. The implication of these studies in understanding the molecular and cellular mechanisms of chronic pain and a potential of certain 5-HT receptor subtypes to become targets of newly developed anti-pain drugs with more properties will be discussed.

This review is a tribute to Sir Hsiang-Tung Chang who left us on November 4, 2007 and to the special issue in memory of Hsiang-Tung Chang. For those who worked with him as a former member of the Shanghai Brain Research Institute and were his students, he was a great scientific personality and the Godfather of the neuroscience and pain research in China.

1 Bidirectional modulation of endogenous descending pain circuits

Individual pain sensation is naturally variable, dependent on subjective experiences except for injury or stimulating intensity. Base on accumulating studies in last three decades, it has been recognized that difference in pain perception may partially depend on the existence of the endogenous pain modulatory systems in the brain [4]. Earlier work was conducted by Melzack and Wall [5] who proposed "gate-control theory" as a model of pain modulatory mechanisms, suggesting that the spinal and medullary dorsal horn receives inputs from both primary nociceptive afferents and local inhibitory neurons and thus plays a critical role in the modulation of pain. In 1964, Tsou and Jang [6] were the first to report that the periaqueductal gray (PAG) of midbrain mediated morphine analgesia in animal. Five years later, local electrical stimulation at the ventrolateral parts of the PAG in midbrain was found to produce anesthesia-like action for gut surgery of rat [7] and to relieve intractable pain in human [8], which soon resulted in identification of endogenous opioidergic inhibitory system including opioid peptides and their binding sites in this region [8–10] and its inhibitory action on spinal nociceptive processing [11]. In addition, blockade of neuronal activity with microinjection of local anesthetic agent lidocaine into the rostroventral medulla (RVM), a medullary reticular area composed by medial nucleus raphe magnus (NRM) and adjacent nucleus reticularis gigantocellularis pars alpha (NGCα), eliminated PAG-evoked antinociception. Also, electrical stimulation or microinjection of morphine in the RVM similarly induces analgesia in rats [12]. As a consequence of electrophysiological, pharmacological and anatomical studies on the structure and function of the RVM, it was identified that the RVM receives signal inputs from PAG neurons including opioidergic projection [13] and is likely to be the final relay in supraspinal influences on spinal nociceptive processing by its descending projections to the spinal dorsal horn [14]. Following these pioneering studies, the concept of descending pain inhibition was established as important to endogenous pain modulation in the 1980s [15]. It has been accepted that endogenous pain modulatory system exists in the form of a descending inhibitory pathway from PAG-RVM circuit to the spinal and medullary dorsal horn, integrating feedback from multiple forebrain areas such as the anterior cingulated cortex, the prefrontal cortex, the insular cortex and the amygdala [4, 16, 17]. This mechanism has been also considered as an important mechanisms underlying contribution of cognitive or emotional influence to change of pain experiences [17].

When descending modulatory pathway is well documented as sources of endogenous inhibitory control for opioid- or stress-mediated analgesia, a serial of electrophysiological studies conducted by Fields and colleagues further facilitated understanding the landscape of descending pain circuitry from the RVM to the spinal dorsal horn [17]. They examined neuronal fire patterns in the RVM paired with the tail-flick, a behavioral nocifensive response to noxious thermal stimulus in lightly anesthetized animals and found that two distinct groups of RVM neurons rapidly exhibited different

changes in state activity just prior to the initiation of the nocifensive withdrawal: ON-cells increased a period of firing and OFF-cells decreased firing [18]. The remaining neurons that failed to correlate with nociceptive stimuli were classified as Neutral-cells. The classifying populations of RVM neurons have been repeatedly confirmed by the distinct pharmacological and neurochemical profiles [19–21]. Both ON- and OFF- cells were found to project to the spinal dorsal horn [20]. Growing evidence supports that the activation of OFF-cells functionally drive descending anti-nociceptive force [22], beginning to lay the ground for a cellular understanding of descending inhibitory modulation. Meanwhile, although there is some controversy about the function of the ON-cells [23, 24], it appears that both reduced activity of OFF-cells and hyperexcitation of ON-cells may exert descending net facilitatory influences that contribute to behavioral hyperalgesia after injury [17, 22], which is consistent with recent appreciation that there is endogenous descending facilitation from the PAG-RVM circuit [25] as mentioned below. In fact, validity of functionally distinct population of RVM neurons has been paralleled by the increased reports of bidirectional descending pain modulation.

Gebhart and colleagues on the first time described bidirectional pain modulation of spinal nociceptive processing from the RVM in early 1990s [26, 27]. Electrical stimulation or administration of glutamate in the RVM induced intensity- or dose-dependent suppression or enhancement of nociceptive responses in spinal neurons and behavioral pain withdrawals, suggesting existence of descending facilitation on nociception [28]. Lesion or general inactivation of RVM neurons was found to attenuate the maintenance of behavioral hyperalgesia induced by tissue or nerve injury, indicating that active descending facilitation is involved in persistent pain [29–31]. Focal application of cholecystokinin, BDNF and proinflammatory cytokines into the RVM results in thermal hyperalgesia [32–34]. Therefore, growing evidence has established the presence of endogenous descending pain facilitation, overlapping with descending inhibitory influences to balance spinal nociceptive modulation and actively involve state-dependent nocifensive behaviors after injury [16, 25, 28, 35–37]. It should be addressed that descending facilitation as an endogenous pain modulatory mechanism provides a biological protective machinery to avoid noxious injury during acute pain states, however long-lasting switch from descending inhibition to facilitation pathologically contributes to maintenance of persistent hyperalgesia and allodynia after tissue and nerve injury. Therefore, translating novel strategies from bench to bedside targeting enhanced descending facilitation will potentially benefit for chronic pain therapy.

2 Bidirectional modulation of descending serotonergic pathway

The increasing appreciation of the RVM-spinal circuit as the final descending pathway mediating bidirectional modulation of nociception and behavioral pain responses has led to the question of its nature of neurochemistry. Neuroanatomical studies reported existence of many neurotransmitters such as 5-HT or GABA and a variety of receptors in RVM neurons projecting to the spinal dorsal horn and presumed to be involved in descending net nociceptive modulation [37]. For example, Electrophysiological evidence indicated that inhibitory GABAergic or glycinergic neurons and mu-opioid receptor (MOR)-expressing neurons in the RVM and their descending projections mediate descending inhibition on spinal pain transmission. Although more than one third of descending projections from the RVM are serotonergic [38], interpreting the role of descending 5-HT in pain modulation proves more challenging due to its distribution in physiologically identified Neutral-cells [39], its anatomical relationship with other molecules in the same cells [37], and the diversity of subtypes of the 5-HT receptors in the spinal and medullary dorsal horn [37, 40]. In recent years, the development of selectively pharmacological tools and genetic manipulation has largely enriched to understand cellular and molecular mechanisms of descending pain modulatory system, in which the important advantage studied is the bidirectional pain modulation of descending 5-HT system, depending on the functional context.

The existence of 5-HT in the brain was first demonstrated in 1953 [41], and majority of 5-HT-containing neurons was mapped in midline raphe nuclei of the brainstem [42]. In mammalian, it is well accepted that the largest aggregate of 5-HT-containing neurons involving in spinal nociceptive modulation are principally located in the RVM and their descending 5-HT fibers form a bulbospinal tract that descends in the dorsolateral funiculi (DLF) of spinal cord and predominately terminates in the spinal dorsal horn [43]. In contrast, 5-HT-containing neurons in midbrain raphe nuclei, mainly

the dorsal and, to a lesser extent, the medial raphe nuclei, provide a diffuse ascending projection to limbic forebrain regions and are important in regulating homeostatic functions and are implicated in the etiology and treatment of mood disorders and schizophrenia [44]. Although there are bidirectional projections from some 5-HT-containing neurons in the dorsal raphe nuclei and other reticular structures, new evidence suggested that the ascending or descending projections of raphe 5-HT neurons are guided by permissive transcript factors encoding homeodomain Hmx^+ or Hox^+ in 5-HT neurons, respectively, during neuronal development [45]. The homeostatic effect of these transcript factors on function of descending 5-HT fibers in adult and their role in pain perception remain to be further examined.

Early evidence suggested that spinal 5-HT release contributes to descending pain inhibition [15]. Besson's group was the first to report that analgesia induced by electrical stimulation of the RVM was accompanied with 5-HT release in spinal cord [46, 47]. Willis and colleagues further found that chemical stimulation of the PAG also induced spinal 5-HT release, along with an obvious analgesia [48]. Intrathecal administration of 5-HT itself evoked antinociception in acute noxious stimulation [49, 50], whereas spinal blockade of 5-HT functions attenuated analgesia induced by intra-RVM electrical stimulation or microinjection of morphine [46, 51, 52]. Furthermore, selective lesion of 5-HT-containing neurons with microinjection of neurotoxin 5, 7-dihydroxytryptamine (5,7-DHT) into the RVM or disruption of the DLF resulted in thermal hyperalgesia [53, 54] or enhanced inflammatory pain states [33]. In addition, *Lmx1b* knockout mice with selective lack of brainstem 5-HT neurons exhibited an amplified behavioral hypersensitivity during acute inflammatory pain [55]. Together, these findings indicate the involvement of active 5-HT neurons in tonic descending inhibition of acute pain and the development of inflammatory pain. It is important to note that multiple neurotransmitter systems have been found to coexist and several functional receptors express in RVM 5-HT-containing neurons [37]. Thus, it is not clear whether the enhanced nociception after deletion of RVM 5-HT-containing neurons or spinal 5-HT-containing fibers is due to the loss of 5-HT action at spinal sites or to the loss of other coexisting inhibitory neurotransmitters, or even to the loss of the integrated effects from multiple receptors expressed in 5-HT-containing neurons. Interestingly, electrophysiological data indicated that 5-HT-containing neurons in the RVM are neither OFF-cells nor ON-cells [56] and may partially belong to Neutral-cells [39, 57]. However, functional MOR expression was mainly found in ON-cells in the RVM [17, 58], whereas nearly half of the spinal projecting 5-HT-containing neurons have been shown to express MOR [59] or to be sensitive to MOR agonists [38]. It seems that at least some descending MOR-expressing ON-cells use 5-HT as a neurotransmitter [60]. Selective chemical deletion of MOR-containing neurons in the RVM was found to attenuate maintenance of neuropathic pain [61]. The selective deletion of MOR-containing ON-cells in the RVM would then reduce descending serotonergic influences. In fact, selective lesion of spinal 5-HT fibers by 5,7-DHT was also reported to reduce central sensitization and attenuates mechanical allodynia after tissue and nerve injury but not thermal hyperalgesia after inflammation [62, 63], suggesting that descending 5-HT pathways may contribute to facilitatory influence on neuronal excitability in the spinal dorsal horn and behavioral hypersensitivity during the development of persistent pain, especially neuropathic pain [40,64]. Given the controversial results in these studies and integrated impairment of descending 5-HT-containing neurons or fibers, the role of descending 5-HT but not 5-HT-containing neurons in the development of thermal and mechanical hypersensitivity after inflammation and nerve injury requires reevaluation. Utilizing combined regional gene targeting with Tph-2 RNA interference to selectively deplete 5-HT from RVM neurons and descending 5-HT-containing axon terminals in the spinal dorsal horn, a new study demonstrated that the RVM 5-HT itself was not involved in tonic descending inhibition and intra-RVM opioid-induced analgesia in acute pain [65]. Importantly, this study identified that the RVM 5-HT system participated in enhanced descending pain facilitation, which was necessary for maintenance but not induction of hyperalgesia and allodynia after inflammation and nerve injury [65]. Thus, some previous results do not demonstrate the integrated effects of 5-HT function in spinal pain processing and modulation because they do not reflect the unique effects of the descending neurotransmitter 5-HT itself. Although 5-HT-containing neurons in the RVM are involved in descending inhibition in acute pain and inflammatory pain but in descending facilitation in neuropathic pain, descending 5-HT signaling itself may be predominately implicated in supraspinal mechanisms

underlying the maintenance of central sensitization and behavioral hypersensitivity after tissue and nerve injury. It is hypothesized that unique functional and structural plasticity of both descending 5-HT-containing neurons and possible spinal 5-HT receptor subtypes may be responsible for a switch in the balance of RVM 5-HT function resulting in enhanced descending facilitation during the development of persistent pain.

3 Bidirectional modulation of spinal serotonergic receptors

It is clear that whether 5-HT can be either inhibitory or excitatory, its effect depends on 5-HT receptor subtype activated [2, 3]. The currently accepted classification of 5-HT receptors (5-HTR) includes seven classes known as 5-HT_1 to 5-HT_7 that comprise 15 subtypes [2, 66]. Most of these receptors at mRNA and protein level have been recently found to present in the spinal dorsal horn. For example, 5-HT_{1D}R, 5-HT_3R, 5-HT_5R and 5-HT_7R are mainly localized in the superficial layer of the dorsal horn [67-71], in which 5-HT_{1D}R and 5-HT_3R are distributed in local cell bodies and dendrites or central terminals of primary afferent fibers; whereas 5-HT_5R is mostly expressed in dorsal horn neurons [68]. Moreover, 15%–20% of 5-HT_7R in the spinal superficial layer was observed in astrocytes [72]. In contrast, 5-HT_{1A}R and 5-HT_{2A}R are highly distributed in the spinal deeper layers and even the ventral horn, respectively [73]. Besides the different laminar distribution, Studies from Yoshimura's group [74, 75] indicated that 5-HTR subtype-specific distribution on excitatory or inhibitory neurons and on local or ascending projection neurons also raise the possibility of a more intimate coordination and balance of descending pain modulation between the activity of descending 5-HT-containing fibers and their target spinal cord circuits. Thus, the presence of many 5-HTR subtypes enables selective drugs to be designed to therapeutically modulate pain processing. For example, 5-HT_7R was found to mainly exist in GABAergic interneurons in spinal lamina III–V and primary afferent terminals in lamina I–II [68, 76]. In addition, it was also reported that half of the GFP-positive GABAergic cells expressed 5-HT_{1A}R but not 5-HT_{2A}R [69]. Furthermore, it has been noted that many central terminals of primary afferents and descending fibers may contain some 5-HTR subtypes. The receptor mRNAs for the 5-HT_{1B}, 5-HT_{1D}, 5-HT_{2A}, 5-HT_3 and 5-HT_7 were detected in the DRG and trigeminal ganglia (TG) neurons by reverse transcriptase polymerase chain reaction technique and in situ hybridization [76–78]; meanwhile, there was no detectable 5-HT_{2C}R expression in the DRG [78, but see 77]. Immunostaining showed that 5-HT_{2A}R was located in small- to medium-sized DRG neurons either binding the isolectin B4 or expressing substance P or TRPV1 receptors [79]. Almost half of 5-HT_{1B}, 5-HT_{1D} or 5-HT_{1F}R-positive DRG and TG neurons express glutamate or CGRP [80], suggesting that synaptic activity of primary afferent fibers could be modulated by descending 5-HT system at the spinal level. Interestingly, very few reports demonstrated the presence of 5-HTR subtypes in the RVM and PAG. At least, 5-HT_{2A}R was also found to be present in the RVM and PAG neurons [81], suggesting that some parts of certain 5-HTR expressions in spinal dorsal horn may drive from descending fibers, besides local neurons and primary afferent inputs. The possible existence of function of 5-HT autoreceptors in presynaptic terminals of descending 5-HT-containing fibers in spinal nociceptive processing need to pay attention and to be further investigated.

The different distribution of 5-HTR subtypes in the spinal dorsal horn and the DRG may result in their different roles in modulation of pain. Although agonists or antagonists are more often used in exploring 5-HTR subtypes function on pain perception and modulation, there are some studies demonstrated that the expression of certain 5-HTR subtypes are regulated after tissue and nerve injury. There was a significant increase in receptor mRNA levels of 5-HT_{1A}, 5-HT_{1B}, 5-HT_{1F}, 5-HT_{2A}, 5-HT_3, 5-HT_4 and 5-HT_7 in the spinal dorsal horn [77, 82, 83] and 5-HT_{1A}, 5-HT_{1B}, 5-HT_{2A} and 5-HT_3 but not 5-HT_{1D}, 5-HT_{1F} and 5-HT_{5A} in DRG tissue [84] after hindpaw inflammation. Increased expression of the 5-HT_{2A}R mRNA was observed in the NRM, the ipsilateral side of ventrolateral PAG and spinal dorsal horn in arthritic rats [81]. Consistently, there were significantly increased protein expressions of 5-HT_{1D}R and 5-HT_{2A}R in the ipsilateral side of spinal cord [85] and the DRG [86] after peripheral inflammation. In the neuropathic pain model, the mRNA of 5-HT_{2C}R was found to be significantly decreased in the ipsilateral spinal cord after trigeminal nerve injury [87]. On the other hand, a significant increase of immunoreactivity of 5-HT_7R [88] and 5-HT_{1D}R [85] was detected in the ipsilateral spinal cord after spinal nerve injury. 5-HT_{2A}R is not significantly different between ipsilateral and controlateral sides of the spinal cord after

nerve injury, although it is increased in inflammatory pain model. Together, tissue and nerve injury produce different pattern of changes in distinct 5-HTR subtype expressions in the spinal dorsal horn and DRG levels, some of which may be involved in descending facilitation and other in descending inhibition, dependent on acute or persistent pain states and targeting areas.

Much endeavor has been intended for understanding the role of functional 5-HTR subtype expression in descending pain modulation, in which electrophysiological and behavioral examination over the last few years has focused on the function of 5-HT_{1A}, 5-HT_2 and 5-HT_3 receptors in spinal synaptic transmission and plasticity as well as nocifensive responses due to available agonists and antagonists selective to these receptors [89]. The results are consistent to behavioral observation that 5-HT_{1A} receptors exert a pronounced inhibitory influence upon nociception, activation of 5-HT_{1A}R mimicked the action of 5-HT and dose-dependently induced outward current in substantia gelatinosa (SG) neurons from *in vitro* the spinal cord of normal rats [90,91]. Also, 5-HT_{1A}R agonist 8-OH-DPAT mimicked the first-phase inhibition of miniature excitatory postsynaptic currents (mEPSCs) and C-afferent-evoked excitatory postsynaptic currents (eEPSCs) induced by 5-HT [92]. Consistently, another study reported that the inhibitory effects of 5-HT and 5-HT_{1A}R agonist on the C-fiber responses of dorsal horn wide dynamic range (WDR) neurons in the spinal cord were significantly decreased following spinal nerve ligation (SNL) [93]. On the contrary, 5-HT_{1A}R agonist was found to potently depress evoked field potentials (FPs) only in SNL-treated rats but not in sham operated rats [94]. Furthermore, methysergide, a nonselective 5-$HT_{1/2}$R antagonist, reversed the suppression of established long-term potentiation (LTP) of C-fiber-evoked FPs in naïve animals and the basal expression of C-fiber-evoked FPs in animal with nerve injury by inhibiting the reuptake of 5-HT [95]. In addition, it was reported that 5-HT was involved in the inhibition of orofacial nociceptive processing via the activation of 5-HT_{1A}R and 5-HT_2R. Application of 5-HT induces a hyperpolarization in the majority of SG neurons of the trigeminal spinal subnucleus caudalis (Vc) in mice and this effects are mimicked by 5-HT_{1A}R and 5-HT_2R agonist and blocked by the antagonists of these two receptors [96]. In contrast, it has been proposed that the activation of 5-HT_{2A}R exerts pronociceptive actions in spinal dorsal horn by sensitizing terminals of peripheral afferent fibers [97,98]. Antagonizing 5-HT_{2A}R blocked 5-HT-induced transient Ca^{2+} signaling in DRG neurons and attenuated mechanical hyperalgesia induced by injection of 5-HT into hindpaw [99]. Blocking spinal 5-HT_{2A}R and 5-HT_{2B}R significantly promotes the depression of C-fiber-evoked spinal FPs by MOR agonist DAMGO in nerve-ligated rats but not in the sham group, indicating that the plastic changes of spinal serotonergic modulation via 5-HT_{2A}R and 5-HT_{2B}R under pathological conditions [100]. Consistently, blockade of 5-HT_{2A}R or 5-HT_{2B}R in spinal cord slices depressed the evoked potentials only after SNL but not in naïve animal, whereas the activation of 5-HT_{2C}R exerts tonic inhibitory activity in both groups [100]. Other study reported the inhibitory actions of spinal 5-HT_{2A}R in synaptic transmission. Both 5-HT_{2A}R and 5-HT_{2C}R antagonists significantly rescued 5-HT-induced inhibition on the C-fiber responses of WDR neurons in normal rats [99]. However, the same effects needed a higher dose of 5-HT_{2C}R antagonist but not 5-HT_{2A}R antagonist in SNL-treated rats.

Numerous studies suggest that 5-HT_3Rs are implicated in descending pain facilitation [60, 71]. Electrophysiological data demonstrated that functional 5-HT_3R was highly expressed in both myelinated and unmyelinated nociceptors and the activation of 5-HT_3R increased release of neurotransmitters from primary afferent terminals into the spinal dorsal horn. In the 5-HT_3R mutant mice, the magnitude of spinal cord neuronal firings during the second phase was significantly reduced after injection of formalin into the center of the receptive field [101]. New behavioral evidence indicated that 5-HT_3R-dependent descending facilitation was mediated by the activation of the protein kinase mammalian target of rapamycin (mTOR)-sensitive pathways, contributing to the maintenance of persistent pain states after nerve injury [102]. Consistently, *in vivo* electrophysiology experiments showed that blockade of spinal 5-HT_3R significantly inhibited stimulation-evoked responses and the inhibitory effects in bone cancer group are much greater than in the sham group, indicating a role for spinal 5-HT_3R-dependent descending serotonergic facilitation in cancer-induced bone pain [103]. However, some studies also shows spinal plasticity does not require the descending serotonergic activity mediated by spinal 5-HT_3R [104]. Furthermore, it was reported that spinal 5-HT_3R may mediate the inhibitory effects of 5-HT on synaptic transmission by activation of GABAergic neurons [74, 75, 90]. The activation of 5-HT_3R

significantly depresses C-fiber-evoked FPs in the spinal cord of both naïve and neuropathic rats [94]. In SG neurons of the spinal cord, bath application of 5-HT or 5-HT_3R agonist increased the amplitude and the frequency of spontaneous inhibitory postsynaptic currents (sIPSCs). Moreover, activation of spinal 5-HT_3R could mimic 5-HT-induced inward current in probably inhibitory neurons [91, 93]. Thus, it seems that most of 5-$HTR_{1–3}$ subtypes exhibit dual effects on normal synaptic transmission or plasticity in the spinal dorsal horn after inflammation and nerve injury. Behaviorally descending inhibition and facilitation will be dependent on net output or state-derived switch of both inhibitory and facilitatory effects from complex circuit and functionally distinct 5-HTR subtype activation in the spinal cord. The function and contribution of spinal diverse 5-HTR subtypes in descending pain modulation and central sensitization underlying the persistent pain states remain to further study.

4 Concluding remarks

Our understanding of neural mechanisms of persistent pain has mainly focused on the primary afferent-derived central sensitization in the spinal dorsal horn. The role of active descending facilitation or reduced descending inhibition in the maintenance of spinal neuronal hyperexcitability and behavioral hypersensitivity after tissue and nerve injury has undergone recent revitalization with the advantage of combination of genetic manipulation, new electrophysiological technique, development of selective receptor agents and behavioral pain assessments. Accumulating evidence suggests that endogenous pain modulation balancing between 5-HT-dependent descending inhibition and facilitation may be mediated by integrated inhibitory or facilitatory influence from functionally distinct 5-HTR subtype activations in the spinal dorsal horn based on acute or chronic pain states. Such inconsistent data and complex observations indicate that there are many open questions on function of descending 5-HT system waiting for clear and precise answers. Appreciating how active descending 5-HT pathways interface with functionally distinct 5-HTR subtypes and subsequently involve maintenance of central sensitization underlying transition of acute pain to persistency after injury will "facilitate" to understand cellular and molecular mechanisms of chronic pain and to develop novel therapies for clinical pain.

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