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# Normal Distribution and Plasticity of Serotonin Receptors after Spinal Cord Injury and Their Impacts on Motor Outputs

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## Abstract

Following spinal cord injury (SCI) a series of anatomical and functional plastic changes occur in the spinal cord, including reorganization of the spinal neuronal network, alteration of properties of interneurons and motoneurons as well as up- or down-regulation of different neurotransmitter receptors. In mammalian spinal cord, one of the important neurotransmitters, serotonin (5-HT), plays an essential role in modulating sensory, motor and autonomic functions. Following SCI, especially complete spinal cord lesion, the descending supply of 5-HT is lost. As a consequence different 5-HT receptors undergo variant degrees of plastic changes.

In this chapter I have systematically reviewed the distribution of different 5-HT receptors in the spinal cord and their plastic changes following SCI where applicable. In addition, the plastic changes of 5-HT supplying system in reaction to SCI have also been reviewed. These results indicate that 5-HT receptors are important factors not only for modulation of normal motor function, their plastic changes are also critical for motor functional recovery and, quite often, for the development of certain pathological states after SCI. Pharmacological and/or genetic intervention of selected 5-HT receptors and/or intrinsic 5-HT producing system in the spinal cord may pave new ways for the restoration of motor functions after SCI.

**Keywords:** monoamine, monoamine receptor, spinal cord, motor control, intraspinal 5-HT cell

## 1. Introduction

Spinal cord injury (SCI) is a devastating condition with an incidence of 10–83 per million people per year worldwide according to statistical data from different countries [1]. It leads to an extensive and usually irreversible loss of sensory functions, voluntary motor control, and autonomic functions below injury level. A variety of primary and secondary complications occur depending on the severity of the injury and time course of its development. These symptoms may involve different systems, manifesting as, e.g., paralysis, spasticity, neuropathic pain, pulmonary and cardiovascular problems, osteoporosis, anemia, pressure ulcers, bladder and bowel problems, and sexual dysfunction [2–8]. Currently, there is no cure for SCI and thus improving quality of life, such as restoration of partial motor and sensory functions, has become a priority for setting up the treatment strategy. The primary cause of these problems is the loss of both descending and ascending projecting pathways in the spinal cord. The descending pathways include direct motor-initiating pathways, such as cortical spinal tracts, and modulatory pathways, such as serotonergic, dopaminergic, and noradrenergic pathways. These monoaminergic systems are so important that in either acute or chronic SCI direct stimulation of the receptors of these monoamines with drugs could regain locomotor activity in animals [9–17]. Thus, restoring the function of monoaminergic systems has become a key strategy to restore motor function and ameliorate secondary symptoms [18–23].

So far a great number of studies have been focusing on the serotonergic system in SCI. In the mammalian spinal cord, serotonin (5-HT) originates mainly in the raphe nuclei of the brainstem and plays an important role in modulating sensory, motor, and autonomic function [24–27]. Following SCI, especially complete spinal cord lesion, the descending supply of 5-HT is lost and as a consequence different 5-HT receptors undergo variant degrees of plastic changes [22, 28–36]. In addition, a potential intraspinal 5-HT-producing system in the spinal cord, i.e., aromatic L-amino acid decarboxylase (AADC) cells, also undergoes plastic changes to increase their potency to produce 5-HT from its precursor [37–40]. Although these plastic changes may induce pathological symptoms such as spasticity and chronic central pain [34, 41, 42], they are essential for spinal function recovery (for review see [43–45]). In this chapter, I will make a systematic review according to up-to-date literature related to the distribution and plastic changes of 5-HT receptors in the spinal cord in normal or SCI states with a note on the intraspinal 5-HT-producing cells.

## 2. 5-HT in the spinal cord in health

In mammals, including humans, 5-HT axons in the spinal cord almost exclusively originate from the brainstem raphe nuclei [46–49]. Their terminals are distributed in all parts of the gray matter at all levels of the spinal cord [50, 51]. The cell bodies with descending 5-HT projections are located in the caudal part of the raphe nuclei, which include the raphe magnus, raphe obscurus, raphe pallidus, ventral lateral medulla, and the area postrema [52]. In the spinal cord, 5-HT projecting fibers descend in the white matter through two different routes: one with

fibers from the raphe magnus in the dorsal part of the lateral funiculus which terminates mainly in the dorsal horn, and the other with fibers from raphe obscurus and raphe pallidus in the ventral funiculus which terminates mainly in the ventral horn and the intermediate zone.

In addition to the descending projecting system, there are indeed 5-HT neurons in the spinal cord although in normal states their contribution of 5-HT can be ignored. So far a small number of intraspinal 5-HT neurons have been reported in macaque monkey (ca. 150 cells per monkey [53]), rat (3–9 cells per rat [54, 55]), and mouse [51]. In the rat spinal cord, 5-HT cells were distributed in different parts of the spinal cord with the exception of cervical segments; in the monkey spinal cord most of the cells were observed in the cervical segments with a small number in other segments; whereas in the mouse spinal cord they were exclusively located in the sacral segments. In the rat spinal cord, the 5-HT cells were found to be located in laminae VII and X in the gray matter, whereas in monkey and mouse spinal cord they were exclusively found in lamina X.

Serotonin in the spinal cord plays an important role in sensory information processing, motor control, and autonomic function. Traditionally, it is hypothesized that 5-HT in the spinal cord exerts its effects by inhibiting sensory systems and facilitating motor systems [56]. However, now it is known that the effects of 5-HT are very complicated both in sensory and motor aspects. In sensory aspect, 5-HT not only has antinociceptive but also pronociceptive effects (e.g., [57, 58]). In motor aspect, except for facilitating motor output, 5-HT also inhibits motor behavior (e.g., [59, 60]). The different functions that 5-HT exert at different circumstances depend on many factors, such as the brainstem origins of the descending projections, the termination localizations of these fibers in the spinal cord, and the activation states of its different receptors.

### 3. 5-HT receptors in the spinal cord in health

The diverse functions of 5-HT in the spinal cord are achieved through the activation of different 5-HT receptors. As seen in **Table 1**, seven families (or types) and at least 14 subfamilies (or subtypes) of 5-HT receptors have been identified so far [61]. In these seven families, with exception of 5-HT<sub>3</sub> receptors that are ligand-gated ion channels, all other families are G protein-coupled receptors [62]. To better understand the diversity of 5-HT functions, it is fundamentally important to have the knowledge of the anatomical localizations of different 5-HT receptors in the spinal cord. A majority, though not all, of 5-HT receptor subfamilies have been found to be expressed in the spinal cord (for reviews see [63, 64]). Here, I will make a systematic review of these receptors in terms of their cellular as well as subcellular localizations in the spinal cord based on available data. One should keep in mind that due to the existence of a great number of splices and editing variants for several 5-HT receptors, a greater degree of operational diversity could be expected, though this issue is not the focus of the present review.

Receptor family (year of molecular gene clone)*	Receptor subfamily (year of molecular gene clone)*	Expression in the spinal cord	Functions at normal states	Expression changes following SCI	Functions at SCI states
5-HT1	1A (1987)	Primary afferent fibers in dorsal horn [66]; neuronal somata in different laminae of gray matter [31,69]; axon initial segments [31,71];	Antinociception [73,76]; pronociception [77]; increases motoneuron excitability [60]; induces central fatigue [81]	Upregulation at least to 30 days after SCI [28,31]	Promotes motor functional recovery [13,14,172]
	1B (1992)	Primary afferent fibers in dorsal horn [83]; neuronal somata at least in intermediate zone [38]	Antinociception [72]; autoreceptor [89]; inhibits activity of AADC cells [38]	--	Inhibits mono- and polysynaptic reflexes [99,174]
	1D (1991)	Primary afferent fibers in dorsal horn [92]; gamma motoneurons in ventral horn [94]	Antinociception, [93]; modulates proprioceptive circuits [94]	--	Inhibits polysynaptic reflex [95]??
	1E (1992)	--	--	--	--
	1F (1993)	Substantia gelatinosa of spinal dorsal horn [98]	Antinociception [101,102]??	--	Inhibits polysynaptic reflex [99]??
5-HT2	2A (1988)	Different laminae of the spinal gray matter but largely in the superficial dorsal horn and lamina IX	Pronociceptive [57,112]; antinociceptive [115]; excites motoneuron [117]; facilitates micturition reflex [119]; facilitates sexual behavior	Upregulation [32,33,35]	Locomotor functional recovery [12,14,43]; 5-HT supersensitivity and muscle spasm [42]

Receptor family (year of molecular gene clone)*	Receptor subfamily (year of molecular gene clone)*	Expression in the spinal cord	Functions at normal states	Expression changes following SCI	Functions at SCI states
		[104,107,108]	[120,121]		
	2B (1992)	Dorsal horn (probably primary afferent fibers) [109]	Pronociception [109,124,126]	Constitutive activation [182]	Motoneuron hyperexcitability and muscle spasm [182]
	2C (1988) (Named 1C before 1993)	Different laminae of the spinal gray matter [36,104,107]	Pronociception [57,112]; antinociception [96,115,133]; induces long-lasting amplification of spinal reflexes [73,134]; inhibits micturition reflex [127,138]	Constitutive activation [34,182]; upregulation [22,36,177]	Motor functional recovery [34,182]; 5-HT supersensitivity, motoneuron hyperexcitability, and muscle spasm [34,42,182]
5-HT3	3A (1991) 3B (1999) 3C (2003) 3D (2003) 3E (2003) [62,139]	Different laminae of the spinal gray matter [107,142]	Antinociception [145,146]; -- pronociception [149]; mediates tail-flick reflexes [117]; inhibits bladder function [152]	--	Promotes motor function recovery [151]
5-HT4 (1995)		Ventral horn, some sympathetic neurons [153]	Pronociception [154]	--	--
5-HT5	5A (1994)  5B (1993)	Dorsal horn, intermediate lateral nucleus, and Onuf's nucleus [110,156] --	Antinociception [58,157]; -- autonomic and micturition control [156] --	-- --	-- --
5-HT6 (1993)		Different laminae of the spinal gray matter [159]	Pronociception [160]	--	--
5-HT7 (1993)		Dorsal horn, intermediate zone, and ventral horn	Pronociception [164–166]; promotes locomotion		Promotes locomotor functional

Receptor family (year of molecular gene clone)*	Receptor subfamily (year of molecular gene clone)*	Expression in the spinal cord	Functions at normal states	Expression changes following SCI	Functions at SCI states
		with a dorsoventral density gradient [16,162]	[16,167,169]		recovery [43,172]

\* Year of molecular gene clone is based on Wikipedia ([https://en.wikipedia.org/wiki/5HT\\_receptor](https://en.wikipedia.org/wiki/5HT_receptor)) except 5-HT3 receptors; --: no data available; ??: data not conclusive.

**Table 1.** 5-HT receptors in the spinal cord and plastic changes following SCI.

### 3.1. 5-HT1 receptors

As listed in **Table 1**, 5-HT1 receptors include five subfamilies: 1A, 1B, 1D, 1E, and 1F. Although all of these receptors have been detected in the brain by different techniques [65], there is no report to my knowledge demonstrating the expression of 5-HT1E receptors in the spinal cord. In addition, the evidence of the existence of 5-HT1F in the spinal cord is mainly from physiological experiments. Nonetheless, I will describe 5-HT1A, 1B, 1D, and 1F in the spinal cord according to the available data.

*5-HT1A receptors:* Data from both autoradiographic (radioligand binding) and immunohistochemical experiments have demonstrated the presence of 5-HT1A receptors in different regions of the spinal gray matter across different spinal segments in rats [31, 66–69]. 5-HT1A receptor binding sites were predominantly seen in the dorsal horn especially laminae I and II [67], where they were partly located in the primary afferent fibers [66]. In autoradiographic images, it is difficult to identify the components located in cell bodies; however, with immunohistochemistry with a 5-HT1A antibody generated from an intracellular epitope, it was clear that 5-HT1A receptors were expressed in cell bodies in the spinal cord [31]. The immunolabeled cell bodies were located in different spinal regions in the gray matter including the dorsal horn, intermediate zone, and ventral horn [31, 69, 70]. In addition, using a different 5-HT1A antibody generated from an extracellular epitope, the receptors were also shown to be present in the axon hillock [31, 71]. There are no studies to investigate 5-HT1A receptor localization at an ultrastructural level and therefore no data available for their subcellular distribution.

It is commonly believed that 5-HT1A receptors exert an inhibitory effect in sensory information transmission including nociception in the spinal dorsal horn (e.g., [72–76]). For example, in mice intrathecal injection of 5-HT1A receptor agonist 8-hydroxy-2-(di-*n*-propylamino) tetralin (8-OH-DPAT) could inhibit the tail-flicks induced by noxious radiant heat [72]. In neonatal rat spinal cord in vitro experiments, Hochman et al. [73] showed that 8-OH-DPAT depressed evoked excitatory postsynaptic potentials (EPSPs) in the deep dorsal horn neurons, whereas 5-HT1A receptor antagonist WAY-100635 (*N*-[2-[4-(2-methoxyphenyl)-1-

piperazinyl]ethyl]-*N*-(2-pyridyl)cyclohexanecarboxamide) facilitated evoked EPSPs. Data from the same group also indicated that the activation of 5-HT<sub>1A</sub> receptors facilitated long-term depression in the dorsal horn neurons. However, the effects of 5-HT<sub>1A</sub> receptors in the dorsal horn are not always inhibitory. For example, the activation of 5-HT<sub>1A</sub> receptors has been reported to facilitate nociceptive [77, 78] and itch transmission [79], and to induce spontaneous tail-flicks [80].

As 5-HT<sub>1A</sub> receptors have been found to be present both at the neuronal somata and the axon initial segments in spinal ventral horn neurons, including motoneurons [31], it is speculated that 5-HT<sub>1A</sub> receptors may have heterogeneous functions on motor outputs, i.e., both excitatory and inhibitory [59, 60]. Indeed, using a turtle spinal cord, slice preparation, and intracellular recording technique, Perrier and Cotel [60] demonstrated that the activation of 5-HT<sub>1A</sub> receptors by 8-OH-DPAT induced an increased excitability in a large fraction (9/11) of sampled motoneurons; whereas in a small fraction of motoneurons (2/11) 8-OH-DPAT gave a hyperpolarizing effect. Subsequent data from the same research group have demonstrated that the inhibitory effect was due to the activation of extrasynaptic 5-HT<sub>1A</sub> receptors located at the axon initial segments, a mechanism supposed to underlie central fatigue [81, 82].

*5-HT<sub>1B</sub> receptors:* To the best of my knowledge, there are no systematic immunohistochemical studies on the distribution of 5-HT<sub>1B</sub> receptors in the spinal cord. Previous studies using autoradiography have demonstrated that 5-HT<sub>1B</sub> receptors did not have any dominant expression region in the spinal gray matter although the intermediate zone seemed to dwell slightly higher labeled profiles [67, 68]. Although it is difficult to differentiate labeled cell bodies from nerve fibers with autoradiography, results from studies using other methods including lesion, pharmacology, and reverse transcription polymerase chain reaction (RT-PCR) indicated the existence of this receptor subfamily both at presynaptic (primary afferent fibers) [83] and postsynaptic locations (motoneurons) [84, 85]. Recently by using immunohistochemistry, Wienecke et al. [38] have found that 5-HT<sub>1B</sub> receptors were indeed expressed in the cell bodies at least in the intermediate zone in the rat spinal cord.

Similar to 5-HT<sub>1A</sub> receptors, a major role of 5-HT<sub>1B</sub> receptors is inhibition in sensory transmission, especially nociception [72, 86, 87]. For example, Eide et al. [72] showed that in mice intrathecal injection of 5-HT<sub>1B</sub> receptor antagonist RU-24969 (5-methoxy-3(1,2,3,6-tetrahydropyridin-4-yl)-1*H*-indole) induced a significant increase of tail-flick latencies. Another role of 5-HT<sub>1B</sub> receptors is to inhibit the release of 5-HT from its fibers via autoreceptor mechanism. Although both 5-HT<sub>1A</sub> and 1B receptors have been demonstrated to be autoreceptors in the brain, in the spinal cord it is most likely that 5-HT<sub>1B</sub> receptors play a more important role than 5-HT<sub>1A</sub> receptors in controlling the release of 5-HT from 5-HT fiber terminals [88–90]. Thus, Brown et al. [89] using rat spinal cord synaptosomes preparation showed that the 5-HT<sub>1B</sub> receptor agonists 1-(*m*-trifluoromethylphenyl)piperazine and 1-(*m*-chlorophenyl) piperazine concentration-dependently decreased [<sup>3</sup>H]5-HT release. In addition, 5-HT<sub>1B</sub> receptors could also inhibit the production of 5-HT from AADC cells in the spinal cord through feed-forward mechanism so that the AADC cells do not produce 5-HT in normal physiological states [38]. Another function of 5-HT<sub>1B</sub> receptors, as noted in mice, is to delay

the maturation of  $\gamma$ -aminobutyric acid (GABA) phenotype in spinal cord during its development [91].

*5-HT<sub>1D</sub> receptors:* Data concerning the anatomical localization of 5-HT<sub>1D</sub> receptors in the spinal cord are relatively poor. The available data indicate that 5-HT<sub>1D</sub> receptors exist both in the dorsal and the ventral horn. Using immunohistochemistry, 5-HT<sub>1D</sub> receptors were found to be expressed in the primary afferent fibers in the superficial dorsal horn of the rat spinal cord [92, 93]. At the ultrastructural level, 5-HT<sub>1D</sub> receptors in the spinal cord dorsal horn were found to be localized exclusively within dense core vesicles of synaptic terminals and not the plasma membrane [92]. Using in situ hybridization and transgenic mouse model, Enjin et al. [94] found that 5-HT<sub>1D</sub> receptors were specifically expressed in  $\gamma$  motoneurons in the ventral horn as well as in some proprioceptive sensory neurons in different spinal regions which were coexpressed with parvalbumin (used as a proprioceptive neuronal marker in the study).

The available data indicate an effect of 5-HT<sub>1D</sub> receptors in the suppression of sensory, especially nociceptive, information transmission [58, 93, 95]. However, different results were also present. For example, in a formalin-induced hindpaw pain model, Jeong et al. [96] showed that the 5-HT<sub>1D</sub> receptor agonist GR-46611 (3-[3-(2-dimethylaminoethyl)-1*H*-indol-5-yl]-*N*-(4-methoxybenzyl)acrylamide) did not suppress the formalin-induced flinching responses and the 5-HT<sub>1D</sub> receptor antagonist BRL-15572 (3-(4-(4-chlorophenyl)piperazin-1-yl)-1,1-diphenyl-2-propanol) failed to reverse the antinociceptive effects of 5-HT. Other functions of 5-HT<sub>1D</sub> receptors include, e.g., depressing the spinal monosynaptic reflex induced by endogenously released 5-HT [97], and shaping proprioceptive circuits to receive and relay accurate sensory information in the spinal cord motor network [94]. In addition, since 5-HT<sub>1D</sub> receptors were expressed in  $\gamma$ , but not  $\alpha$ , motoneurons, they could be used as a marker to identify  $\gamma$  motoneurons in the spinal cord [94].

*5-HT<sub>1F</sub> receptors:* To my knowledge, there is no systematic investigation concerning the expression of 5-HT<sub>1F</sub> receptors in the spinal cord of any species. However, there are indeed some pieces of evidence from studies using radioligand or physiological technique indicating that this receptor subfamily is present in the spinal cord dorsal horn. Castro et al. [98] used [<sup>3</sup>H]sumatriptan as a radioligand in the presence of suitable concentrations of 5-carboxamidotryptamine (5-CT) to define 5-HT<sub>1F</sub> receptors in the human spinal cord, and they found significant levels of binding sites in substantia gelatinosa in the cervical spinal cord. However, because 5-CT is not a specific 5-HT<sub>1F</sub> agonist, the data cannot be taken as granted. Using an in vitro sacrocaudal spinal cord preparation from spinal transected rats, Murray et al. [99] showed that the EPSPs and associated long polysynaptic reflexes were consistently inhibited by 5-HT<sub>1F</sub>-specific agonist LY-344864 (*N*-[(3*R*)-3-(dimethylamino)-2,3,4,9-tetrahydro-1*H*-carbazol-6-yl]-4-fluorobenzamide), indicating the existence of 5-HT<sub>1F</sub> in the spinal cord. However, the exact locations of these receptors are yet to be determined.

Due to the lack of related anatomical data, it is difficult to unveil the functions of 5-HT<sub>1F</sub> in the spinal cord. Considering that 5-HT<sub>1F</sub> receptors are expressed in the trigeminal and spinal dorsal root ganglia [100] and their agonists have been used to treat migraine [101, 102], it is most likely that this receptor subfamily exerts an antinociceptive effect in spinal cord.

### 3.2. 5-HT2 receptors

As listed in **Table 1**, 5-HT2 receptors include three subfamilies, i.e., 5-HT2A, 2B, and 2C. Before 1993, 5-HT2C receptors were named 5-HT1C receptors [103]. By using different experimental techniques including autoradiography, in situ hybridization, Western blot and immunohistochemistry all these three receptor subfamilies have been detected in the spinal cord in different species [104–109] although 5-HT2C subfamily is probably not expressed in cat spinal cord [105]. 5-HT2A and 2C receptors are likely among the most thoroughly investigated 5-HT receptors in the spinal cord due to their important functions in both sensory information processing and motor control.

*5-HT2A receptors:* Using various techniques, the existence of 5-HT2A receptors has been confirmed at both mRNA and protein level in the spinal cord of different species [104–108, 110]. By using polymerase chain reaction (PCR) together with Southern hybridization technique, Helton et al. [105] showed that spinal cord tissues from rat, cat, monkey, and human contained 5-HT2A receptor mRNAs. By using in situ hybridization, Pompeiano et al. [104] showed that 5-HT2A receptor mRNAs were present at intermediate density in the ventral horn. This distribution pattern has been confirmed with immunohistochemistry [106, 108]. Doly et al. [108] systematically investigated the distribution of 5-HT2A receptor immunoreactivity in rat spinal cord and the results indicated that 5-HT2A receptors were distributed in all the spinal segments with a similar immunolabeling pattern. The most prominent labeling was found in lamina IX, where many, but not all, motoneurons were labeled. At lumbar five to six level, 5-HT2A receptors were densely expressed in Onuf's nucleus that contains the motoneurons innervating pelvic striated muscles controlling sexual behavior and micturition [110]. Another region with dense immunolabeling was lamina II. The remaining laminae of the gray matter displayed a weak to moderate labeling. Doly et al. [108] also examined the subcellular localization of 5-HT2A receptors in laminae II and IX and they demonstrated that most immunolabeled profiles were postsynaptic, i.e., dendrites and cell somata, although a small number of axons and axon terminals were also labeled. The immunoreactive product was localized mainly on the plasma membrane where synaptic specifications were lacking.

The localization of 5-HT2A receptors in the neurons in both the spinal dorsal and ventral horn endows their functions in both sensory information transmission and motor control. For the sensory information modulation, evidence to date largely favors in their pronociceptive role [57, 111–114]. For example, Kjærsvik et al. [112] showed that in formalin-induced pain model, intrathecal injection of DOI ((±)-2,5-dimethoxy-4-iodoamphetamine hydrochloride), a 5-HT2A/2C receptor agonist, augmented nociceptive response in rats. This effect could be completely abolished when ketanserin (a 5-HT-2A receptor antagonist) was coadministered. Rahman et al. [57] investigated the effects of DOI, ketanserin, and ritanserin (another 5-HT2A/2C antagonist) on the evoked responses of dorsal horn neurons to electrical, mechanical, and thermal stimulation, and found that the activation of 5-HT2A receptors facilitated the spinal nociceptive transmission under normal physiological states. However, there is also evidence indicating that 5-HT2A receptors exert an antinociceptive effect [115, 116]. For example, using a rat spinal cord slice preparation Xie et al. [115] found that bath application of 5-HT increased the frequency of spontaneous inhibitory postsynaptic currents in GABAergic and

glycinergic neurons in substantia gelatinosa. TCB-2 (4-bromo-3,6-dimethoxybenzocyclobuten-1-yl)methylamine hydrobromide), a 5-HT<sub>2A</sub> receptor agonist, could mimic the 5-HT effect, and ketanserin could partially inhibit the effect of 5-HT and completely inhibit the effect of TCB-2.

It is not surprising that 5-HT<sub>2A</sub> receptors play a significant role in motor control considering their intensive expression in ventral horn motoneurons. Indeed, direct stimulation of 5-HT<sub>2</sub> receptors in motoneurons in vivo with selective 5-HT<sub>2</sub> receptor agonist DOI or DOM (2,5-dimethoxy- $\alpha$ ,4-dimethylbenzene ethamine hydrochloride) produced dose-related back muscle contractions and wet dog shakes which could be markedly attenuated by ritanserin, ketanserin, or mianserin, suggesting an effect from 5-HT<sub>2A</sub> receptors [117]. Their direct facilitating effect on spinal motoneurons was also evidenced by application of DOI in rat spinal cord in vitro experiments [118]. In addition, pharmacological experiments showed that 5-HT<sub>2A</sub> receptor activation facilitated micturition reflex and activated the external urethral sphincter in female rats [119], whereas in both male and female rats 5-HT<sub>2A</sub> receptors seemed to involve sexual behavior [120, 121].

*5-HT<sub>2B</sub> receptors:* In comparison with 5-HT<sub>2A</sub> and 5-HT<sub>2C</sub>, 5-HT<sub>2B</sub> receptors in the spinal cord have been less investigated and the available data are inconsistent. Using in situ hybridization expression of 5-HT<sub>2B</sub> receptors was not found in rat spinal cord and even brain [104]. However, using several other techniques, the receptors have been detected in the spinal cord. Thus, using reverse transcription polymerase chain reaction (RT-PCR) 5-HT<sub>2B</sub> receptor mRNAs have been detected in spinal cord tissue in many different species including rat, cat, monkey, and human [105]. Using microarray global gene expression technique [122] and immunohistochemistry [123], 5-HT<sub>2B</sub> receptors have been detected in the rat spinal cord motoneurons. In addition, using Western blot it was shown that the receptor proteins were also present in the dorsal part of the spinal cord [109]. Considering that with Western blot and immunohistochemistry 5-HT<sub>2B</sub> receptors have been demonstrated to be expressed in the dorsal root ganglia [109, 124], their expression in the spinal dorsal horn is likely originating from the primary afferent fibers from the dorsal root ganglia, although it cannot be excluded that they might be also expressed in neuronal somata (e.g., [125]).

It is presumable that 5-HT<sub>2B</sub> receptors are largely related with sensory information transmission considering their localization in dorsal root ganglia and dorsal horn. Thus, evidence from a few studies related to 5-HT<sub>2B</sub> receptor functions in the spinal cord favors that the receptors are responsible for facilitating mechanical hyperalgesia, tactile allodynia, and nociception [109, 124, 126]. Studies concerning the receptors' motor function are rare. One study showed that 5-HT<sub>2B</sub> receptors likely increased urethral smooth muscle tone since the 5-HT<sub>2B</sub> receptor antagonist RS-127445 (3-(4-(4-chlorophenyl)piperazin-1-yl)-1,1-diphenyl-2-propanol) blocked increase in urethral pressure in female rats [127]. In frog, the activation of 5-HT<sub>2B</sub> receptors by  $\alpha$ -methoxy-5-HT (a 5-HT<sub>2B</sub> receptor agonist) facilitates N-methyl-D-aspartate (NMDA)-induced depolarization of motoneurons [128]. In addition, 5-HT<sub>2B</sub> receptors seemed to modulate respiratory activity in rats [123].

*5-HT<sub>2C</sub> receptors:* 5-HT<sub>2C</sub> receptor mRNAs and proteins were widely distributed in different laminae of the spinal gray matter including motoneurons as demonstrated by different

experimental methods, such as PCR [105], autoradiography [68, 129], in situ hybridization [104, 107], and immunohistochemistry [36, 130–132]. Using PCR combined with Southern hybridization 5-HT<sub>2C</sub> receptor mRNAs were detected in spinal cord tissue from rat, monkey, and human, but not in cat spinal cord [105]. By using in situ hybridization, Fonseca et al. [107] showed that 5-HT<sub>2C</sub> receptor mRNAs were present at high levels in most parts of the spinal gray matter, except lamina II. Using immunohistochemistry, Ren et al. [36] have investigated 5-HT<sub>2C</sub> receptor expression in both normal and spinalized rats and the results showed that 5-HT<sub>2C</sub> receptors were widely distributed in different regions of the spinal gray matter (except lamina II) and were predominantly located in the neuronal somata and their dendrites although they also seemed to be present in axonal fibers in the superficial dorsal horn. Thus, the data from in situ hybridization and immunohistochemistry fit very well for this receptor subfamily.

The wide distribution of 5-HT<sub>2C</sub> receptors in different laminae of the spinal cord endows the receptors' role both in sensory information transmission and motor control. For the sensory information transmission available data point to that 5-HT<sub>2C</sub> receptors play roles both in pronociception [57, 112] and antinociception [96, 115, 133]. For their role on pronociception, refer to the part concerning 5-HT<sub>2A</sub> receptors since most studies used DOI (a common agonist for 5-HT<sub>2A</sub> and 2C receptors) to stimulate and ketanserin to inhibit the receptors in response to different nociceptive stimulations. However, because ketanserin is mainly a 5-HT<sub>2A</sub> receptor antagonist some researchers argued that the pronociception effect should preferably attribute to 2-HT<sub>2A</sub> than 2C receptors. Their effects on antinociception seem to be adequately evidenced. For instance, in one study Jeong et al. [96] showed that in formalin-induced pain model 5-HT<sub>2C</sub> receptor specific antagonist D-MC (*N*-ormethylclozapine/8-chloro-11-(1-piperazinyl)-5*H*-dibenzo[*b,e*][1,4]diazepine) could block the suppression effect of 5-HT, whereas 5-HT<sub>2C</sub> receptor specific agonist MK-212 (6-chloro-2-(1-piperazinyl)pyrazine hydrochloride) could suppress the formalin response. In another study, Xie et al. [115] showed that a 5-HT<sub>2C</sub> receptor agonist WAY-161503 (8,9-dichloro-2,3,4,4a-tetrahydro-1*H*-pyrazino[1,2-*a*]quinoxalin-5(6*H*)) mimicked the 5-HT antinociceptive effect in dorsal horn neurons and this effect could be blocked by a 5-HT<sub>2C</sub> receptor antagonist, *N*-desmethylclozapine.

In motor control, one of the functions of 5-HT<sub>2C</sub> receptors is the induction of a long-lasting amplification of spinal reflex [73, 134–136]. Machacek et al. [134] showed that, in an in vitro neonatal rat spinal cord preparation, superfusion of 5-HT depressed reflex responses recorded in the ventral roots which was induced by electrical stimulation of primary afferents. However, following 5-HT washout, a long-lasting reflex facilitation was observed. Further pharmacological analysis indicated that it was the activation of 5-HT<sub>2C</sub> but not 5-HT<sub>2A</sub> receptors that was required for this long-lasting reflex. Although 5-HT<sub>2C</sub> receptors play a major role in long-term motor reflexes, they also play an inhibitory role in some physiological states. For example, in mice, when activated by DOI, 5-HT<sub>2C</sub> receptors inhibited the locomotor activity which opposed the effects of 5-HT<sub>2A</sub> receptors [137]. In addition, 5-HT<sub>2C</sub> receptors were also demonstrated to inhibit the micturition reflex [119, 127, 138]. Thus, Conlon et al. [138] showed that, in guinea pigs, Ro-600175 (( $\alpha$ S)-6-chloro-5-fluoro- $\alpha$ -methyl-1*H*-

indole-1-ethanamine fumarate), a 5-HT<sub>2C</sub> receptor agonist, increased peak urethral pressure in a dose-dependent manner. This effect was reversed by a selective 5-HT<sub>2C</sub> receptor antagonist SB-242084 (6-chloro-2,3-dihydro-5-methyl-*N*-[6-[(2-methyl-3-pyridinyl)oxy]-3-pyridinyl]-1*H*-indole-1-carboxamide) but not a 5-HT<sub>2A</sub> or 2B receptor antagonist. Similar results were also observed in rats [119, 127]. Clinically, duloxetine, a serotonin-norepinephrine reuptake inhibitor, has been used to treat stress urinary incontinence. One possible mechanism for this effect may be the activation of 5-HT<sub>2C</sub> receptors in motoneurons in Onuf's nucleus, which leads to an increased activity of pudendal motor neurons and a subsequent increase in the strength of urethral sphincter contractions.

### 3.3. 5-HT<sub>3</sub> receptors

Although genes encoding the molecular structures of five different 5-HT<sub>3</sub> receptor subfamilies (A–E) have been cloned, it is demonstrated so far that only the 5-HT<sub>3A</sub> subfamily is functional in homomeric form, and all other subfamilies require coassembling with 5-HT<sub>3A</sub> as functional heteromers [62, 139]. Therefore, here 5-HT<sub>3</sub> receptors will be described together as one receptor unity. In the spinal cord, 5-HT<sub>3</sub> receptors in the beginning were detected mainly in the dorsal horn with autoradiography and immunohistochemistry in rats and humans [140, 141]. Later by using in situ hybridization and immunohistochemistry, they were found to be expressed in different laminae across the spinal gray matter [107, 142]. By using immunohistochemistry, Morales et al. [142] observed labeled cell bodies in the dorsal horn among the densely labeled fiber terminals. In the ventral horn, large neurons, likely motoneurons, were densely labeled. By using in situ hybridization, Fonseca et al. [107] found that 5-HT<sub>3</sub> receptor mRNAs were expressed in different laminae in the rat spinal cord with very low levels in the dorsal horn, slightly higher levels in laminae VI through X and the highest level in lamina IX. Maxwell et al. [143] have studied the ultrastructure of 5-HT<sub>3</sub> immunolabeled profiles in the dorsal horn of the rat spinal cord. They found that 5-HT<sub>3</sub>-immunopositive terminals invariably formed asymmetric synaptic junctions with dendritic profiles and often contained a mixture of granular and agranular vesicles. Immunoreactive cells were found to contain intense patches of reaction product within their cytoplasm. Although there are no data available regarding the subcellular morphology of 5-HT<sub>3</sub> receptors in the ventral horn neurons, from the light microscopic data, it can be concluded that the receptor immunoreactive product was located at least in the cytoplasm [142].

It has long been known that 5-HT<sub>3</sub> receptors modulate spinal nociceptive reflexes [144]. A large part of literature demonstrated a role of 5-HT<sub>3</sub> receptors in antinociception in the spinal cord (e.g., [145, 146]). This is likely due to, for instance, that the receptors interact with inhibitory interneurons such as GABA interneurons in the dorsal horn [147]. However, there are also data indicated that 5-HT<sub>3</sub> receptors have a pronociceptive effect. For example, Guo et al. [148] showed that when the spinal 5-HT<sub>3</sub> receptors were activated by intrathecal injection of a selective 5-HT<sub>3</sub> receptor agonist SR-57227 (1-(6-chloropyridin-2-yl)piperidin-4-amine) spinal glial hyperactivity, neuronal hyperexcitability, and pain hypersensitivity were induced in rats. These diverse effects could be explained by, e.g., the expression of 5-HT<sub>3</sub>

receptors in different neuronal components and/or the different effects of assemblies formed from different receptor subfamilies [149, 150].

Although 5-HT<sub>3</sub> receptors are expressed in the ventral horn neurons data concerning their motor functions are scarce. A study by Guertin and Steuer [151] showed that in hindlimb paralyzed mice 5-HT<sub>3</sub> receptor agonist SR-57227 could produce hindlimb movements although with a low score. This result indicates that 5-HT<sub>3</sub> receptors might also modulate spinal motoneuron activity in normal states. In addition, the activation of 5-HT<sub>3</sub> receptors by 2-methyl-5-HT, another 5-HT<sub>3</sub> receptor agonist, could induce sideward tail-flick reflex in rats [117], which might likely involve both sensory and motor components in the spinal cord. Further, 5-HT<sub>3</sub> receptors have been reported to inhibit micturition in cats in both normal and spinalized situation [152].

### 3.4. 5-HT<sub>4</sub> receptors

Data concerning 5-HT<sub>4</sub> receptors in the spinal cord are scarce. As far as I know, so far only one article reported the expression of 5-HT<sub>4</sub> receptors in the spinal cord [153]. Using immunohistochemistry, Suwa et al. [153] investigated 5-HT<sub>4</sub> receptor cell distribution in the brain and spinal cord in juvenile rats and found a high density of immunostained neurons in the ventral horn of the spinal cord. In addition, several sympathetic neurons were also seen to be immunopositive.

Similarly, studies about the functions of 5-HT<sub>4</sub> receptors in the spinal cord are also rare. Using pharmacological method, Godínez-Chaparro et al. [154] showed that 5-HT in the spinal cord promotes the development and maintenance of secondary allodynia and hyperalgesia caused by formalin stimulation via the activation of 5-HT<sub>4/6/7</sub> receptors. Considering the anatomical distribution of 5-HT<sub>4</sub> receptors in the spinal cord, they should also play a role in some motor functions. Whether this is the case needs to be investigated further.

### 3.5. 5-HT<sub>5</sub> receptors

The 5-HT<sub>5</sub> receptor family comprises two members: 5-HT<sub>5A</sub> and 5-HT<sub>5B</sub>. Although 5-HT<sub>5B</sub> receptors have been found in some structures in rat and mouse brain, they are not expressed in humans [155]. Therefore, I will only describe the 5-HT<sub>5A</sub> receptors in this chapter.

5-HT<sub>5A</sub> receptors were first identified in the rat spinal cord by Doly et al. [156]. They showed that in the rat spinal cord, 5-HT<sub>5A</sub> receptors were expressed with high density in the superficial dorsal horn (laminae I and II) especially in lamina II. In addition, they were also expressed in other regions including the intermediolateral nucleus in the thoracolumbar region and Onuf's nucleus in lumbosacral region. Ventral horn motoneurons in different regions were weakly labeled. Subcellularly, the receptors were found both in the cytoplasm and on the cell membrane of neuronal somata and dendrites. In the cytoplasm, they were associated with Golgi apparatus, rough endoplasmic reticulum, and vesicles. On the membrane, they were exclusively located on the postsynaptic density that was in clear contrast with the subcellular localization of 5-HT<sub>2A</sub> receptors [108, 110].

According to their locations in the spinal cord, it is speculated that 5-HT<sub>5A</sub> receptors are related to spinal modulation of pain, autonomic function, and control of micturition. Indeed, data from recent pharmacological studies have demonstrated that 5-HT-induced antinociceptive effect was mediated by spinal 5-HT<sub>5A</sub> receptors in several pain models, such as that induced by formalin, capsaicin, or acetic acid [58, 157]. For example, injection of 5-HT or 5-CT (an agonist of 5-HT<sub>5A</sub> receptors) could dose-dependently prevent nociception induced by formalin [157]. However, the speculated effects of the receptors on autonomic function and control of micturition yet need to be demonstrated.

### 3.6. 5-HT<sub>6</sub> receptors

Using RT-PCR, Gérard et al. [158] discovered the existence of 5-HT<sub>6</sub> receptor mRNAs at a moderate level in the rat spinal cord. Later, using immunoautoradiogram, data from the same group showed that 5-HT<sub>6</sub> receptor immunoreactive product seemed to be present across all laminae of the spinal gray matter with a denser labeling in the superficial dorsal horn and lamina IX [159]. No data are available about the subcellular distribution of 5-HT<sub>6</sub> receptors in the spinal cord. However, according to the electron microscopic data from other brain regions such as the striatum and the hippocampus, 5-HT<sub>6</sub> receptors were mainly associated with postsynaptic dendrites and no immunopositive axon terminals were found [159].

The exclusive roles of 5-HT<sub>6</sub> receptors in the spinal cord yet remain to be elaborated. Using a formalin pain model in rats, Castañeda-Corral et al. [160] suggested that 5-HT<sub>6</sub> receptors play a pronociceptive role in the spinal cord since intrathecal injection of EMD-386088 (5-chloro-2-methyl-3-(1,2,3,6-tetrahydro-4-pyridinyl)-1*H*-indole), a selective 5-HT<sub>6</sub> receptor agonist, enhanced formalin-induced nociception. It is unknown whether 5-HT<sub>6</sub> receptors play any role in motor control.

### 3.7. 5-HT<sub>7</sub> receptors

As there were no specific radioligands for 5-HT<sub>7</sub> receptors available, data concerning their distribution in the spinal cord acquired using autoradiography had been inconclusive [161] until 2005 when Doly et al. [162] used immunohistochemistry to study their distribution in the rat spinal cord. Doly et al. [162] showed that in the rat lumbar spinal cord 5-HT<sub>7</sub> receptors were mainly located in two superficial laminae of the dorsal horn. Except in the Onuf's nucleus where the labeling was relatively denser, immunolabeling in the ventral horn motoneuron region was generally weak. At subcellular level, the receptors were found in the postsynaptic locations in neuronal cell bodies and dendrites as well as presynaptic locations in unmyelinated and thin myelinated axonal fibers. In addition, immunolabeling was also found in astrocytes. In cats, Noga et al. [16] found that in thoracolumbar spinal cord 5-HT<sub>7</sub>-immunolabeled cells spread across different laminae in the gray matter with a dorsoventral density gradient.

Available data showed that 5-HT<sub>7</sub> receptors exert multiple roles in modulating both sensory and motor behavior. In sensory aspect 5-HT<sub>7</sub> receptors have been demonstrated to exert dual but diverse actions in nociception depending on the different situations. In healthy rats, 5-HT<sub>7</sub>

receptor agonists exerted a pronociceptive action but in neuropathic animals they exerted an antinociceptive action [163]. Yesilyurt et al. [164] showed that intrathecal application of SB-269970 ((2*R*)-1-[(3-hydroxyphenyl)sulfonyl]-2-(2-(4-methyl-1-piperidinyl)ethyl)pyrrolidine), a 5-HT<sub>7</sub> receptor antagonist, blocked both opioid and nonopioid type stress-induced analgesia, and this effect was mediated by descending serotonergic pathways and the spinal 5-HT<sub>7</sub> receptors. Several pieces of evidence have indicated that the nonopioid analgesic drug Nefopam also took its effects via activation of 5-HT<sub>7</sub> receptors [165, 166].

5-HT<sub>7</sub> receptors have been demonstrated to be critical for 5-HT-induced locomotor-like activity [16, 167–169]. Liu and Jordan [167] showed that in rats 5-HT<sub>7</sub> receptor antagonists blocked locomotor-like activity induced by stimulating mid-medulla region and also decreased step cycle duration. These results have been verified in 5-HT<sub>7</sub> knockout mice [169]. Considering that 5-HT<sub>7</sub> receptor antagonists blocked locomotor-like activity when applied only above the L3 segments [167], it is possible that they activated the central pattern generator neurons above this level that expressed 5-HT<sub>7</sub> receptors and responded to 5-HT stimulation. Indeed, in decerebrated cats in which locomotion was induced by electrical stimulation of the mesencephalic locomotor region abundant c-Fos immunoreactive cells were observed in laminae VII and VIII throughout the thoracolumbar segments [16].

#### **4. Expression changes of different 5-HT receptors and their significance in motor outputs following SCI**

Following SCI the descending 5-HT projections are interrupted and as a consequence, a number of 5-HT receptors undergo different degrees of plastic changes below the lesion depending on the severity of injury, which on one hand will facilitate the re-establishment of neuronal circuits in the spinal cord below the lesion and thus promote functional recovery, and on the other hand will result in pathological symptoms. These changes can occur at both anatomical and functional levels. However, due to the fact that more functional than anatomical data are available, for some receptors only functional changes have been reported. So far 5-HT receptors that have been confirmed to undergo anatomical changes include, but are not limited to, 5-HT<sub>1</sub> and 5-HT<sub>2</sub> receptor families. In some other receptors, functional (activity) changes are indeed detected although there are no data revealing their anatomical changes. This group of receptors includes 5-HT<sub>3</sub> and 5-HT<sub>7</sub> receptors. So far there are no reports as to the anatomical or functional changes for 5-HT<sub>4</sub>, 5-HT<sub>5</sub>, and 5-HT<sub>6</sub> receptors following SCI. Below I will mainly describe the 5-HT receptors that have been clearly demonstrated to undergo anatomical and/or functional changes in relation to their impact on motor outputs.

##### **4.1. 5-HT<sub>1</sub> receptors**

Among the different 5-HT<sub>1</sub> subfamilies, 5-HT<sub>1A</sub> receptors were mostly investigated in terms of their anatomical and functional changes probably due to their direct involvement in functional recovery following SCI. For other subfamilies, such as 5-HT<sub>1B</sub>, 1D, and 1F, related

studies were less abundant and results relating to their roles in motor functional outputs were also less conclusive.

*5-HT1A receptors:* Using autoradiography, the expression of 5-HT1A receptors have been shown to increase in the rat spinal cord 3 weeks following destruction of descending serotonergic fibers with 5,7-dihydroxytryptamine [83]. In cats, using the same technique, it was found that after spinal cord transection at T13 level binding density was significantly increased in laminae II, III, and X of lumbar segments at 15 and 30 days, but at 60 days the binding density recovered to the control level [28]. It should be addressed that using binding technique it is difficult to differentiate whether the labeled profiles were from neuronal cell bodies, their dendrites or axon fiber terminals. Using immunohistochemistry with two different 5-HT1A antibodies, one labeling neuronal somata and the other labeling the axon initial segments, Otoshi et al. [31] reported that 8 weeks following complete spinal transection at T7-T8 level the expression of 5-HT1A receptors in axon hillock was increased in laminae III, IV, VII, and IX, whereas the expression in neuronal somata and dendrites were increased in laminae VII and IX. This upregulation, both in the axon hillock and neuronal somata and dendrites, was dependent on the sensory input since the receptors were not upregulated when the spinal cord was isolated. Using in situ hybridization, Cornide-Petronio et al. [170] saw a similar upregulation time course for 5-HT1A receptors in lampreys as in cats. They reported an acute upregulation of 5-HT1A receptors in the spinal cord after SCI both in the rostral and caudal part of the lesion site, and the upregulation recovered to normal levels at 3 weeks. This quick recovery of 5-HT1A receptor expression may be due to that in lamprey serotonergic descending projecting neurons in the rhombencephalon could regenerate their axons across the lesion site after complete spinal cord transection [171]. Thus, the variation of results from these studies may partly reflect the different receptor responses to SCI in different species.

There is ample evidence that the activation of 5-HT1A receptors could induce motor functional recovery after SCI [13, 84, 172]. Antri et al. [13] found that, in thoracic spinal cord transected rats, after daily systemic application of 5-HT1A receptor agonist 8-OH-DPAT, locomotor function was significantly improved when compared with control spinalized animals. The agonist had both short- and long-term effects for motor functional improvement. A similar result was also demonstrated by Jackson and White [84] that in acute C1 spinal cord transected rats, after intravenous administration of 8-OH-DPAT, the excitability of spinal motoneurons was markedly enhanced. However, local application of 8-OH-DPAT directly into the ventral horn by microiontophoresis inhibited the glutamate-evoked firing of motoneurons. These results indicate that when 8-OH-DPAT was directly applied into the vicinity of the motoneurons it may activate 5-HT1A receptors in the axon hillock which inhibits the motoneuron firing [81]. However, the marked increase in firing of motoneurons induced by systemic administration of 8-OH-DPAT suggests that 5-HT1A receptors in other locations, likely at sites presynaptic to the motoneurons, have also been activated. In addition to the functions described above, 5-HT1A receptors have been reported to increase bladder capacity under saline or acid infused conditions in SCI cats [173].

*5-HT1B receptors:* In comparison with 5-HT1A receptors, studies concerning plastic changes of 5-HT1B receptors following SCI are sparse. Using autoradiography, Laporte et al. [83]

reported that when descending serotonergic projections were destroyed by 5,7-dihydroxytryptamine the labeling of 5-HT<sub>1B</sub> receptors was decreased (−12%) in the dorsal horn at the cervical but not at the lumbar level in rats. Interestingly, when noradrenergic systems had been lesioned by DSP-4 (*N*-(2-chloroethyl)-*N*-ethyl-2-bromobenzylamine) there was an increase of labeling of 5-HT<sub>1B</sub> receptors both at the cervical level (+31%) and the lumbar level (+17%). In a C2 hemisection rat model, using quantitative RT-PCR, Mantilla et al. [86] did not detect expression changes of 5-HT<sub>1B</sub> receptors in phrenic motoneurons 2 or 3 weeks following the injury.

Similar to their functions in normal physiological states, in SCI states 5-HT<sub>1B</sub> receptors also play an inhibitory role for motor outputs possibly through inhibitions on both mono- and polysynaptic reflexes. In acute C1 spinalized rats, Honda et al. [174] showed that serotonergic depression of monosynaptic reflex transmission induced by application of 5-HTP was mediated by 5-HT<sub>1B</sub> receptors. In S2 spinal cord transection model (also called tail spasticity model), Murray et al. [99] showed that polysynaptic EPSPs that trigger muscle spasms after SCI were inhibited by 5-HT<sub>1B</sub> receptors.

*5-HT<sub>1D</sub> and 1F receptors:* To my knowledge, no studies have investigated the expression changes of these two receptors following SCI. Functionally, the activation of 5-HT<sub>1D</sub> receptors seemed to reduce sensory transmission in both monosynaptic and polysynaptic reflexes in humans [95], and to inhibit bladder activity in cats after SCI [175]. The activation of 5-HT<sub>1F</sub> seemed to inhibit long polysynaptic reflexes in rats after SCI [99]. However because the agonists used in these studies were not specific enough for selected receptors the results were not conclusive.

## 4.2. 5-HT<sub>2</sub> receptors

5-HT<sub>2</sub>, especially 5-HT<sub>2A</sub> and 2C, receptors are the most intensely studied receptors among all 5-HT receptor families mostly due to their considerable effects on the motor functional recovery and perhaps also on the pathological symptoms developed following SCI such as spasticity. Due to the close relationship and similar functions of these three receptor subfamilies, their influences on the motor outputs after SCI will be described together.

*5-HT<sub>2A</sub> receptors:* Using different SCI animal models such as contusion, hemisection, and complete spinal transection, it is confirmed that 5-HT<sub>2A</sub> receptors underwent different degrees of plastic changes following SCI. In thoracic contusive rats using immunohistochemistry, Lee et al. [30] found a moderate yet significantly increased 5-HT<sub>2A</sub> receptor expression (~1.2-fold of control) in the motoneurons at L5-L6 level after 4 weeks of injury. With the same method in a C2 hemisection rat model Fuller et al. [29] demonstrated a significant upregulation (~1.7-fold of control) of 5-HT<sub>2A</sub> receptors in the ipsilateral phrenic motoneurons after 2 weeks of injury. In a S2 spinal transection model, Kong et al. [32, 33] reported a dramatic increase of 5-HT<sub>2A</sub> receptor immunoreactivity (~5.6-fold of control) in the motoneurons below the lesion. The upregulation began as early as 1 day after injury, reached a maximal level by 28 days and lasted at least until 60 days, the longest time interval investigated. Similarly an upregulation of 5-HT<sub>2A</sub> receptors were also observed in chronic (6 weeks) thoracic spinal transected rats. Navarrett et al. [35] reported a ~1.3-fold increase of 5-HT<sub>2A</sub>

receptor mRNAs in the spinal tissue (including both white and gray matter) below the lesion with RT-PCR. Upregulation of 5-HT<sub>2A</sub> receptor mRNAs was also reported in mice subjected to an acute thoracic spinal transection. Thus, Ung et al. [176] found that 3 h after the lesion 5-HT<sub>2A</sub> receptor mRNA expression levels were increased 2.5-fold in the lateral intermediate zone in L1-L2 segments and they remained to be elevated for at least 2 weeks. However, no significant change was found in the ventral horn motoneuron regions, which was strikingly different from that in the rat.

*5-HT<sub>2B</sub> receptors:* No expression changes have been reported for this receptor subfamily in the spinal cord following SCI. With microarray global gene analysis technique, Wienecke et al. [122] did not detect significant changes of 5-HT<sub>2B</sub> receptor mRNAs in S2 spinal transected rats.

*5-HT<sub>2C</sub> receptors:* Similar to 5-HT<sub>2A</sub> receptors, 5-HT<sub>2C</sub> receptor expression changes have been investigated in different SCI animal models with different techniques. However, discrepancies exist as to whether 5-HT<sub>2C</sub> receptors are upregulated or not following SCI. In chronic SCI rats (~15 weeks) severe contusion or transection at thoracic level (T9) could induce a significant increase of 5-HT<sub>2C</sub> receptor immunoreactivity in lumbar spinal cord [22]. The increase could reach 4–7-fold in the ventral horn and ~17-fold in the dorsal horn over control level. In thoracic (T8-T9) spinal transected neonatal rats, following 4 weeks injury 5-HT<sub>2C</sub> receptor immunoreactivity was seen to increase 4–5-fold both in the spinal dorsal and ventral horn [177]. In a S2 spinal transection rat model, data from our group [36] indicated that 5-HT<sub>2C</sub> receptor immunoreactivity increased in all parts of the spinal gray matter below the lesion from 2 weeks but did not reach a significant level until 3 weeks (~1.4-fold over control animals). The increase sustained thereafter and a maximal level was reached at 45 days (~1.7-fold) and maintained at 60 days, the longest investigated interval. It is somehow perplexing that although upregulation was observed at protein level no significant increase at mRNA level was detected in different SCI animal models [34, 35, 122, 178]. However, although no significant changes of the total amount of 5-HT<sub>2C</sub> receptor mRNAs were detected, Murray et al. [34] did detect certain constitutive isoforms, such as INI isoform, that were significantly increased in the motoneurons 6 weeks after S2 spinal transection.

*Functional significance for motor outputs of 5-HT<sub>2</sub> receptors:* The activation of 5-HT<sub>2</sub> receptors has different effects on motor outputs after SCI, which include promoting motor functional recovery and causing maladaptive pathological motor symptoms such as spasticity. Due to uncertainty of the expression of 5-HT<sub>2B</sub> receptors in the spinal cord most studies have been focused on 5-HT<sub>2A</sub> and 2C receptors. There is ample evidence for the roles of 5-HT<sub>2A</sub> and 2C receptors in motor functional recovery following SCI from different studies (e.g., [9, 12, 14, 34, 43, 84, 134, 176, 179, 180], for reviews see [44, 45]). For example, in thoracic spinalized cats, when a 5-HT<sub>2A/C</sub> receptor agonist (e.g., quipazine or DOI) was administered  $\alpha$ -motoneuron excitability was increased and hindlimb motor activity was enhanced [9]. Similar results were also demonstrated in SCI rats [12, 14] and mice [176]. Although 5-HT<sub>2A</sub> and 2C receptors both play a common role for motor functional recovery each subfamily may also exert different functions in different aspects of the motor outputs. Using different agonists and antagonists specific for each of these two receptor subfamilies it is found that 5-HT<sub>2A</sub> receptors might contribute to locomotor network activation and locomotor-like movement generation

[15, 43, 176]. Direct stimulation of 5-HT<sub>2C</sub> receptors with its agonist meta-chlorophenylpiperazine improved weight-supported locomotion in adult rats spinalized as neonates [177, 181]. 5-HT<sub>2C</sub>, probably also 2B, but not 2A, receptors could become constitutively active which could contribute to motor functional recovery following SCI [34, 182].

One maladaptive consequence for 5-HT<sub>2</sub> receptor plastic changes is the induction of muscle spasm due to increased motoneuron excitability. The rapid and robust upregulation of 5-HT<sub>2A</sub> receptors in spinal motoneurons might be responsible for 5-HT supersensitivity after SCI [32, 33]. Activation of 5-HT<sub>2C</sub> receptors could induce long-lasting reflexes of motoneurons in the *in vitro* spinal cord preparations [134, 135]. These long-lasting reflexes could be enhanced when 5-HT<sub>2C</sub> receptors became constitutively active following SCI [34, 182]. One of the cellular mechanisms for the increased motoneuron excitability is that the activation of 5-HT<sub>2</sub> receptors could enhance calcium and/or sodium persistent inward currents [42, 183].

In addition to promoting locomotion, 5-HT<sub>2</sub> (especially 2A) receptors also play a significant role for respiratory functional recovery after cervical spinal hemisection [179] and in enhancing bladder function in thoracic spinal transected rats [184].

It should be addressed that the upregulation of 5-HT<sub>2</sub> receptors does not by all means lead the motoneurons toward a hyperexcitatory state. Recently one interesting finding is presented by Bos et al. [185] who showed that the activation of 5-HT<sub>2A</sub> receptors could increase K(+)-Cl(-) cotransporter (KCC2) expression on cell membrane in the spinal cord motoneurons after SCI. The upregulation of KCC2 receptors in turn increased the activity of GABA A and glycine receptors which would restore endogenous inhibition and reduce spasticity after SCI in rats [186]. These results indicate that the upregulation of 5-HT<sub>2A</sub> receptors would not simply produce an excitatory effect. Rather, their activation will trigger an inhibitory factor which attempts to balance the excitatory effect. This effect seems to be paradoxical to the 5-HT<sub>2A</sub> receptors' direct excitatory effect on motoneurons. However, this might be one of the common functional mechanisms of a spinal network—different factors interact and compensate each other, and the final motor output is determined by a summed vector of these push-pull forces acted on the motoneurons. In fact, any motor action cannot be arisen solely from the activation of a single factor; instead it needs a concert activity of multiple factors including both excitatory and inhibitory. For instance, Hayashi et al. [22] have showed that in contusive SCI rats, application of 5-HT<sub>1A</sub> or 5-HT<sub>2C</sub> receptor agonists alone or in combination could not improve hindlimb motor function; rather, motor functional improvement could be achieved only when the 5-HT precursor 5-HTP was administrated, indicating that simultaneously multiple 5-HT receptor activations are needed for motor functional recovery. This concept is reinforced by the results from Noga et al. [16] who showed that in paralyzed, decerebrated cats, when locomotion was induced by electrical stimulation of the mesencephalic locomotor region, most locomotor-activated cells, labeled with c-Fos, colocalized with 5-HT<sub>1A</sub>, 5-HT<sub>2A</sub>, and 5-HT<sub>7</sub> receptors in laminae VII and VIII in the thoracolumbar spinal region.

### 4.3. 5-HT<sub>3</sub> receptors

There are no data demonstrating the expression changes of 5-HT<sub>3</sub> following SCI. Laporte et al. [83] did not detect 5-HT<sub>3</sub> receptor expression changes with binding technique in rat spinal cords whose descending serotonergic projections had been destroyed by 5,7-dihydroxytryptamine. However, lesions in peripheral nerves could reduce 5-HT<sub>3</sub> receptor expression in the spinal motoneurons in rats [187].

Most studies concerning 5-HT<sub>3</sub> receptor functions have been focused on sensory aspects, especially nociception. Studies relating to their functions in motor outputs after SCI are scarce. So far there is only one study having investigated the influence of the activation of 5-HT<sub>3</sub> receptors on motor outputs in SCI mice. In this study, Guertin and Steuer [152] showed that in hindlimb paralyzed mice 5-HT<sub>3</sub> receptor agonist SR-57227 could produce hindlimb movements although with a low score. This result, anyhow, provided the first evidence that 5-HT<sub>3</sub> receptors could modulate spinal motoneuron activity after SCI.

### 4.4. 5-HT<sub>7</sub> receptors

So far there are no data available with respect to the expression changes of 5-HT<sub>7</sub> receptors following SCI. However, the activation of 5-HT<sub>7</sub> receptor has been shown to be related to locomotor functional recovery in SCI states [23, 43, 172]. Landry et al. [172] showed that in thoracic spinal transected mice systemic application of 8-OH-DPAT, an agonist for both 5-HT<sub>1A</sub> and 5-HT<sub>7</sub> receptors, acutely induced hindlimb movements with characteristics similar to normal locomotion. When the animals were pretreated with SB-269970, a selective 5-HT<sub>7</sub> receptor antagonist, 8-OH-DPAT-induced movements were reduced. Sławińska et al. [23] showed that, in thoracic spinal transected rats, grafting of neurons from the B1, B2, and B3 descending 5-HT system from the brainstem into the spinal cord below the lesion effectively restored coordinated plantar stepping, and the application of SB-269970 disrupted the inter- and intralimb coordination. Further evidence from the same group indicated that 8-OH-DPAT facilitated plantar stepping in chronic spinalized rats [43]. Since 5-HT<sub>7</sub> receptor agonists mainly improved coordination movement the authors assumed that 5-HT<sub>7</sub> receptors mainly facilitated the activity of the central pattern generator interneurons. This assumption is supported by anatomical data that 5-HT<sub>7</sub> receptors were mainly expressed in the neurons in the dorsal horn and the intermediate zone of the spinal gray matter [16]. In addition to the effects on locomotion, it has been reported that the activation of 5-HT<sub>7</sub> receptors by their agonist LP44 (4-[2-(methylthio)phenyl]-*N*-(1,2,3,4-tetrahydronaphthalen-1-yl)-1-piperazinehexanamide) increased bladder voiding efficiency in chronic thoracic spinal transected rats [188].

## 5. Plasticity of 5-HT innervations after SCI

It is evidenced that a small number of 5-HT receptors, or more precisely receptor isoforms, could become active following SCI without ligand activation—so-called constitutive activation. However, for most of the 5-HT receptors that do not have constitutive isoforms the

presence of their ligand—5HT is a necessity for their activation. Then, where could 5-HT originate following SCI? After contusion spinal cord injury or partial spinal cord transections there are still spared supraspinal serotonergic projections and these serotonergic fibers could undergo plastic changes to eventually supplement lost 5-HT supply in the injured spinal cord. For example, in contusion injury the density of spared 5-HT fibers below the lesion varies depending on the locations along the dorsoventral axis and the severity of the injury [22, 189, 190], and 5-HT innervation could be partially restored with time which is responsible at least partly for motor functional recovery [190]. In thoracic hemisection injury, the density of 5-HT fibers in the ipsilateral ventral horn varied from 8% to 30% of the control value according to studies with a postinjury interval 4–7 days [39, 191–194]. The data are inconsistent as to whether 5-HT fibers increase with time following hemisection. Some researchers reported a gradual increase of 5-HT fibers on the ipsilateral side below the lesion (e.g., [193, 195]), whereas some others did not see apparent changes (e.g., [39, 194]). For example, Saruhashi et al. [193] observed that 5-HT-immunoreactive fibers recovered from 20% to about 75% of the normal value in the ventral horn during first 4 weeks. Camand et al. [195] also reported a similar finding in the intermediate zone. However, Filli et al. [194] reported a decrease to about 10% by 4 weeks from about 30% at day 4 in ventral horn motoneuron region. Azam et al. [39] reported that by 60 days the density of 5-HT-immunoreactive fibers in the intermediate zone was reduced to 11% from 23% at 5 days, whereas in the ventral horn it was not reduced (23% vs. 22%). Following complete spinal transection, some 2–15% of the normal content of 5-HT remained in the spinal cord below the lesion ([196]; for review see [25]).

The next question would be then where the residual 5-HT originates following complete spinal transection. One origin might be the intraspinal serotonergic neurons. However, intraspinal serotonergic neurons are very few in number and sparsely distributed (see Section 1). Then, what are the other possible sources? Recent findings from our [38] and Bennett's group [37] indicated that AADC cells in the spinal cord might be another origin. AADC is an essential enzyme for the synthesis of 5-HT, dopamine, and certain trace amines from their respective precursors. AADC cells are widely distributed in different regions of the spinal cord [38], not limiting to the area around the central canal as reported previously [197]. Following SCI the ability of AADC cells in the spinal cord to synthesize 5-HT/dopamine from 5-HTP/L-dopa was dramatically increased and 5-HT/dopamine produced in the AADC was responsible for the increased motoneuron ability recorded both in vivo and in vitro [37, 38, 40, 198]. Nonetheless, we have to admit that without 5-HTP application 5-HT could not be detected in the spinal AADC cells. This might be due to that the detecting techniques used in related studies were not sensitive enough to disclose a small amount of 5-HT, or the turnover rate of 5-HT produced in the AADC cells was so high that once produced it was immediately metabolized. One piece of evidence to support the speculation that monoamine transmitters could be produced from AADC cells in the spinal cord after SCI comes from Hou et al. [199], who showed that the number of dopamine-producing cells, which contained both AADC and tyrosine hydroxylase, was increased in lumbosacral spinal cord in thoracic spinal transected rats; and with enzyme-linked immunosorbent assay (ELISA) dopamine with a content of about 10% of its normal value could be detected in this part of the spinal cord even without L-dopa applica-

tion. At the same time they also showed that dopamine produced from the spinal cord below the lesion was implicated for micturition functional recovery following SCI.

## 6. Conclusions

The serotonin system in the spinal cord is an important modulator for sensory, motor as well as autonomic function. This system normally exercises its function via the interaction between ligand 5-HT and a number of 5-HT receptors expressed in different structures in the spinal cord. Among the 14 5-HT receptor subfamilies at least 12 have been detected in the spinal cord, in which 5-HT<sub>1A</sub>, 2A, 2C, and 7 receptors have been demonstrated to be more important for motor control. In normal physiological states, these receptors coordinate with each other and also with other monoamine systems to enable smooth and controllable motor outputs for our physical activity [200]. Following SCI extensive plastic changes occur for a number of 5-HT receptor subfamilies, mostly with increased receptor numbers and/or constitutive activity. In addition, plasticity also occurs for descending serotonergic fibers (in the case of incomplete SCI) and intraspinal serotonin-producing cells. The coeffects of these plastic changes, together with the plastic changes of other monoamine systems, ultimately lead to increased motoneuron excitability in the chronic phase [17, 21, 108]. These plastic changes have both beneficial and detrimental effects for spinal motor outputs after SCI. Although the plastic changes can result in an adaptive compensation of the lost transmitters and thus assist in motor functional recovery, they can also result in a plethora of maladaptive problems, such as spasticity. In clinical practice, a strategy needs to be set so as to maximally take advantage of the positive side of the plasticity to promote motor functional recovery and meanwhile to reduce negative effects to a minimal extent. To reach this endpoint, pharmacological and/or genetic interferences need to be utilized so that the activity of different 5-HT receptors and 5-HT supply in the spinal cord reach a new balance and consequently an appropriate motor behavior is generated. Such a strategy would certainly have great implications for future treatment of SCI patients.

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## Abbreviations

5-CT: 5-carboxamidotryptamine

5-HT: 5-hydroxytryptamine, serotonin

8-OH-DPAT: 8-hydroxy-2-(di-*n*-propylamino) tetralin

AADC: aromatic L-amino acid decarboxylase

BRL-15572: 3-(4-(4-chlorophenyl)piperazin-1-yl)-1,1-diphenyl-2-propanol

C, T, L, and S: cervical, thoracic, lumbar, and sacral spinal cord, respectively

D-MC: *N*-ormethylclozapine/8-chloro-11-(1-piperazinyl)-5*H*-dibenzo[*b,e*][1,4]diazepine

DOI: ( $\pm$ )-2,5-dimethoxy-4-iodoamphetamine hydrochloride

DOM: 2,5-dimethoxy- $\alpha$ ,4-dimethyl-benzene ethamine hydrochloride

DSP-4: *N*-(2-chloroethyl)-*N*-ethyl-2-bromobenzylamine

ELISA: enzyme-linked immunosorbent assay

EMD-386088: 5-chloro-2-methyl-3-(1,2,3,6-tetrahydro-4-pyridinyl)-1*H*-indole

EPSP: excitatory postsynaptic potential

GABA:  $\gamma$ -aminobutyric acid

GR-46611: 3-[3-(2-dimethylaminoethyl)-1*H*-indol-5-yl]-*N*-(4-methoxybenzyl)acrylamide

KCC2: K(+)-Cl(-) cotransporter

L-dopa: L-3,4-dihydroxyphenylalanine

LP44: 4-[2-(methylthio)phenyl]-*N*-(1,2,3,4-tetrahydronaphthalen-1-yl)-1-piperazinehexanamide

LY-344864: *N*-[(3*R*)-3-(dimethylamino)-2,3,4,9-tetrahydro-1*H*-carbazol-6-yl]-4-fluorobenzamide

MK-212: 6-chloro-2-(1-piperazinyl)pyrazine hydrochloride

mRNA: messenger ribonucleic acid

NMDA: *N*-methyl-D-aspartate

PCR: polymerase chain reaction

Ro-600175: ( $\alpha$ S)-6-chloro-5-fluoro- $\alpha$ -methyl-1*H*-indole-1-ethanamine fumarate

RS-127445: 3-(4-(4-chlorophenyl)piperazin-1-yl)-1,1-diphenyl-2-propanol

RT-PCT: reverse transcription polymerase chain reaction

RU-24969: 5-methoxy-3(1,2,3,6-tetrahydropyridin-4-yl)-1*H*-indole

SB-242084: 6-chloro-2,3-dihydro-5-methyl-*N*-[6-[(2-methyl-3-pyridinyl)oxy]-3-pyridinyl]-1*H*-indole-1-carboxamide

SB-269970: (2*R*)-1-[(3-hydroxyphenyl)sulfonyl]-2-(2-(4-methyl-1-piperidinyl)ethyl)pyrrolidine

SCI: spinal cord injury

SR-57227: 1-(6-chloropyridin-2-yl)piperidin-4-amine

TCB-2: 4-bromo-3,6-dimethoxybenzocyclobuten-1-yl)methylamine hydrobromide

WAY-100635: *N*-[2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl]-*N*-(2-pyridyl)cyclohexanecarboxamide

WAY-161503: 8,9-dichloro-2,3,4,4a-tetrahydro-1*H*-pyrazino[1,2-*a*]quinoxalin-5(6*H*)

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