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Review Article

# Serotonergic mechanisms in spinal cord injury

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ABSTRACT

Spinal cord injury (SCI) is a tragic event causing irreversible losses of sensory, motor, and autonomic functions, that may also be associated with chronic neuropathic pain. Serotonin (5-HT) neurotransmission in the spinal cord is critical for modulating sensory, motor, and autonomic functions. Following SCI, 5-HT axons caudal to the lesion site degenerate, and the degree of axonal degeneration positively correlates with lesion severity. Rostral to the lesion, 5-HT axons sprout, irrespective of the severity of the injury. Unlike callosal fibers and cholinergic projections, 5-HT axons are more resistant to an inhibitory milieu and undergo active sprouting and regeneration after central nervous system (CNS) traumatism. Numerous studies suggest that a chronic increase in serotonergic neurotransmission promotes 5-HT axon sprouting in the intact CNS. Moreover, recent studies in invertebrates suggest that 5-HT has a pro-regenerative role in injured axons. Here we present a brief description of 5-HT discovery, 5-HT innervation of the CNS, and physiological functions of 5-HT in the spinal cord, including its role in controlling bladder function. We then present a comprehensive overview of changes in serotonergic axons after CNS damage, and discuss their plasticity upon altered 5-HT neurotransmitter levels. Subsequently, we provide an in-depth review of therapeutic approaches targeting 5-HT neurotransmission, as well as other pre-clinical strategies to promote an increase in re-growth of 5-HT axons, and their functional consequences in SCI animal models. Finally, we highlight recent findings signifying the direct role of 5-HT in axon regeneration and suggest strategies to further promote robust long-distance re-growth of 5-HT axons across the lesion site and eventually achieve functional recovery following SCI.

## 1. Introduction

Spinal cord injury (SCI) is a devastating event that not only causes physical and emotional anguish to affected individuals but also presents our society with important socio/economic burdens (Ackery et al., 2004). Worldwide prevalence of SCI ranges between 236 and 1298 patients per million and its estimated annual incidence is 8 to 246 cases per million globally (Furlan et al., 2013). Clinical signs associated with SCI depend on both the anatomical level of the injury (cervical to sacral) and lesion severity, extending from minor sensory/motor/autonomic impairments to complete quadriplegia. Although recent advances highlight that epidural electrical stimulation combined with activity-based therapy can improve motor function in a few SCI patients (Angeli et al., 2014; Formento et al., 2018; Wagner et al., 2018), the widespread clinical application of this approach awaits further study. Currently, there is no effective treatment to promote long-distance axon regeneration across the lesion site, restore appropriate connections and establish sustained functional recovery after SCI. Following SCI, there is

*Abbreviation:* AD, Alzheimer's disease; BBB, Basso, Beattie and Bresnahan locomotor scale; BDNF, Brain derived neurotrophic factor; BMS, Basso mouse scale; BS-NSC, Embryonic brainstem-derive neural stem cell; CNS, Central nervous system; CPG, Central pattern generator; CSF, Cerebrospinal fluid; CSPGs, Chondroitin sulfate proteoglycans; E, Embryonic; EMG, Electromyographic; GAP43, Growth-associated-protein-43; GFAP, Glial fibrillary acidic protein; hENPs, Human embryonic neural progenitors; hMPC, Human mesenchymal precursor cell; ISP, Intracellular sigma peptide; Kdyn, Kilodynes; MAG, Myelin-associated glycoprotein; MAO-A, Monoamine oxidase A; MCs, Mesenchymal cells; NgR, Nogo-66 receptor; NT-3, Neurotrophin-3; mTOR, Mammalian target of rapamycin; NPs, Neural precursors; OMgp, Oligodendrocyte-myelin glycoprotein; PCPA, P-chloroamphetamine; PST, Polysialyltransferase; PTEN, Phosphatase and tensin homolog; SCs, Schwann cells; SCI, Spinal cord injury; SERT, Serotonin re-uptake transporter; shRNA, Short hairpin RNA; Trp, Tryptophan; TPH, Tryptophan hydroxylase; tph-1, Tryptophan hydroxylase 1; VIM, Vimentin; 5-HT, 5-Hydroxytryptamine, serotonin; 5-HIAA, 5-Hydroxyindole acetic acid; 5-HTPDC, 5-Hydroxytryptophan decarboxylase; 5HTR, 5-HT receptors; 5-HTP, 5-Hydroxytryptophan

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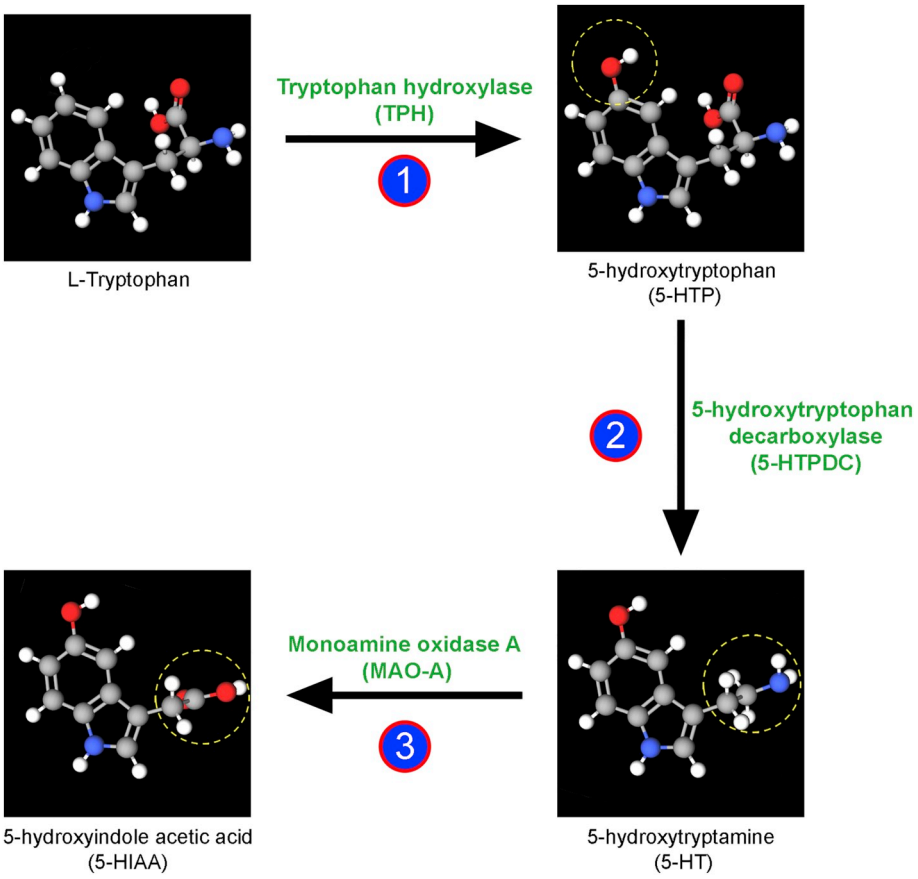


**Fig. 1.** Serotonin biosynthesis and metabolism. 5-HT is synthesized through two enzymatic steps: (1) the addition of a hydroxyl group to the aromatic amino acid l-tryptophan (TrP) by tryptophan hydroxylase (TPH) forming 5-hydroxytryptophan (5-HTP), and (2) side chain decarboxylation of 5-HTP by 5-hydroxytryptophan decarboxylase (5-HTPDC). Subsequently, 5-HT is metabolized by monoamine oxidase A (MAO-A) giving rise to 5-hydroxyindole acetic acid (5-HIAA).

an irreversible loss of sensory, motor, and autonomic functions caudal to the lesion site due to regeneration failure of both ascending and descending axons. Ascending axons originate from dorsal root ganglia and transmit sensory information from the periphery to the central nervous system (CNS). Descending axons of the spinal cord originate from multiple brain regions, including: the motor cortex (corticospinal tract), the locus coeruleus (noradrenergic), the diencephalon (dopaminergic), and the raphe nuclei of the brainstem (serotonergic), the latter being the primary focus of this review (Bowker et al., 1981; Proudfit and Clark, 1991; Qu et al., 2006).

Serotonin, also termed 5-hydroxytryptamine (5-HT), plays a prominent role in locomotion [for recent reviews see (Gackière and Vinay, 2014; Ghosh and Pearse, 2014; Nardone et al., 2015)] and increased 5-HT neurotransmission promotes functional recovery after SCI (Gimenez y Ribotta et al., 1998a; Gimenez y Ribotta et al., 1998b; Hains et al., 2001; Yakovleff et al., 1995). Unlike callosal fibers and cholinergic projections of the basal forebrain and the brainstem, 5-HT axons display better resistance to inhibitory environments after CNS insult, and undergo compensatory sprouting in transgenic mouse model of Alzheimer's disease (AD) (Harkany et al., 2001; Hawthorne et al., 2011; Noristani et al., 2011; Noristani et al., 2010; Zhou et al., 1995). Furthermore, recent studies in invertebrates demonstrate a direct role of 5-HT in promoting axonal regeneration after injury (Alam et al., 2016; Chen et al., 2011). The effect of 5-HT is mediated by activation of specific receptors [for review see, (Nichols and Nichols, 2008)], of which there are seven classes, including six G-protein coupled receptors (5-$HTR_{1,2,4–7}$) and one ligand gated cation channel (5-$HTR_3$) (Nichols and Nichols, 2008).

In this review, we briefly discuss the initial imperative studies leading to 5-HT discovery followed by a short description of 5-HT biosynthesis and neurotransmission, the projection of serotonergic axons into the spinal cord, as well as the physiological involvement of 5-HT in regulating motoneuron and bladder function. Next, we present a detailed examination of serotonergic axon plasticity following CNS traumatism and altered neurotransmitter level. In addition, we summarize therapeutic strategies focusing on 5-HT neurotransmission, and their functional impact in animal models of SCI. Furthermore, we discuss recent studies suggesting a pro-regenerative effect of 5-HT in injured axons. Lastly, we suggest future research directions not only to better understand molecular mechanisms responsible for the unique 5-HT neuronal response after CNS traumatism, but also to stimulate 5-HT axonal crossing of the lesion site and establishing robust long-distance functional re-growth following SCI.

## 2. Central 5-HT neurotransmission

### 2.1. Discovery

Two independent laboratories made seminal contributions to 5-HT discovery and characterization, over 82 years ago. Initially in Rome, Italy, in 1937, Maffu Vialli and Vittorio Erspamer (1909–1999) identified a substance derived from enterochromaffin cells of the gut causing smooth muscle contraction in the rat uterus (Vialli and Erspamer, 1937). They originally named this substance "*Enteramine*" (Vialli and Erspamer, 1942). Subsequently, in the late 1940s in Cleveland Ohio, USA, Maurice Rapport (1919–2011), Arda Green (1899–1958), and Irvine Page (1901–1991) purified 5-HT as a "*serum vasoconstrictor*" and devised the name "*serotonin*" due to its initial purification from the serum ("*sero-*") and its modulation of the vessel tone ("*-tonin*") (Rapprot et al., 1948a, b) [see also (Göthert, 2013)]. Shortly afterwards, in 1949, Rapport elucidated the chemical structure of serotonin as 5-hydroxytryptamine (Rapprot, 1949). By 1952, Erspamer and his colleague Biagio Asero confirmed that 5-HT and enteramine were indeed the same substance (Erspamer and Asero, 1952).

Within a year, Betty Twarog (1927–2013), John Welsh (1901–2002) and Irvine Page reported 5-HT presence in the brain (Twargo and Page, 1953), and suggested its function as a neurotransmitter (Welsh, 1953).

### 2.2. Biosynthesis and synaptic transmission

In mammalian CNS, 5-HT is synthesized through two successive enzymatic steps, involving (1) the addition of a hydroxyl group to the aromatic amino acid L-tryptophan (TrP) (Fig. 1, step 1), followed by (2) the decarboxylation of 5-hydroxytryptophan (5-HTP) (Nichols and Nichols, 2008) (Fig. 1, step 2). Subsequently, 5-HT is packaged into synaptic vesicles, transported to axon terminals and undergoes $Ca^{2+}$-dependent exocytosis (Ruddick et al., 2006). 5-HT-mediated effects are terminated by its pre-synaptic re-uptake via the serotonin re-uptake transporter (SERT) or its post-synaptic metabolism by monoamine oxidase A (MAO-A), giving rise to 5-hydroxyindole acetic acid (5-HIAA) (Fig. 1, step 3).

### 2.3. 5-HT Neurons

Although the first anatomical description of the dorsal raphe (the major site of 5-HT neurons) was made in the early 1900s by Santiago Ramón y Cajal (1852–1934), (Ramón y Cajal, 1904), it was not until 1964 that two Swedish Neurobiologists, Annica Dahlström (1941 – Present) and Kjell Fuxe (1938 – Present) elucidated the anatomical distribution of 5-HT neurons. Using fluorescent immunohistochemistry, Dahlström and Fuxe identified 9 clusters of 5-HT-containing neurons within the midline raphe nuclei of the brainstem, which they classified alphanumerically as B1 – B9 neurons (Dahlstrom and Fuxe, 1964). The serotonergic nuclei (B1 – B9) of the brainstem were subsequently subdivided into rostral (B4 – B9) and caudal (B1 – B3) groups (Jacobs and Azmitia, 1992; Törk, 1990). The rostral group, with their soma located in the midbrain and rostral pons, include the nucleus pontins central oralis (B4), the median raphe nucleus (B5), the dorsal raphe nucleus (B6 – B7), the caudal linear nucleus (B8), and the medial lemniscus (B9), that collectively project towards the forebrain and the brainstem (Jacobs and Azmitia, 1992; Rodríguez et al., 2011; Vertes and Crane, 1997). The caudal group, with their soma in the medulla oblongata, include the nucleus raphe pallidus (B1), the raphe obscurus (B2), the nucleus raphe magnus (B3), the area postrema and the lateral medulla, all giving rise to descending 5-HT projections directed towards the cerebellum and the spinal cord (Jacobs and Azmitia, 1992; Törk, 1990) (Fig. 2A). It is important to note that though B1 – B9 nuclei are defined by their shared ability to synthesize 5HT, more recent findings highlight molecular and functional diversity between 5-HT synthesizing neurons (Okaty et al., 2015). Specifically, 5-HT neurons display marked differences in their global gene expression profiles, depending on their anatomical domain and developmental lineage (Okaty et al., 2015). In addition, 5-HT neurons within the same raphe nuclei also show important differences in their electrophysiological properties (Okaty et al., 2015).

### 2.4. 5-HT innervation of the spinal cord

Caudal 5-HT projections terminate at specific subfields in a non-overlapping manner at all levels of the spinal cord (Fig. 2B). The raphe magnus (B3) predominantly gives rise to the dorsal 5-HT projections that innervate laminae I and II of the dorsal horn (Bowker et al., 1981; Bowker et al., 1982). 5-HT projections within the ventral horn arise from the raphe obscurus (B2), whereas the raphe pallidus (B1) forms the intermediate 5-HT projections that mostly innervate the intermediate zone of the spinal cord (Bowker et al., 1982; Törk, 1990). This pattern of 5-HT spinal cord innervation is generally comparable between mammalian species, including rodents, non-human primates, and humans (Liang et al., 2015; Marlier et al., 1991; Perrin et al., 2011; Ridet et al., 1993). Over 60% of the dorsal 5-HT projections do not form typical synapses, suggesting volumetric transmission, i.e. the neurotransmitter diffuses from release sites through the extracellular space before reaching its high affinity receptor targets (Hentall et al., 2006; Marlier et al., 1991; Ridet et al., 1993). On the other hand, the ventral and intermediate 5-HT projections form classical axo-dendritic and axo-somatic synapses with motoneurons (Privat et al., 1988). In mice during development, descending 5-HT axons initially appear in the cervical spinal cord by embryonic day 12.5 (E12.5) and further extend towards thoracic and lumbar segments by E14.5 and E16.5, respectively (Ballion et al., 2002; Xia et al., 2017). The ventral and intermediate 5-HT projections also become evident by E16.5, whilst the dorsal 5-HT projections are not visible until post-natal day 0 in mice (Ballion et al., 2002). Functionally, 5-HT neurotransmission in the spinal cord modulates sensory, motor and autonomic functions. 5-HT also modulates other neurotransmitter systems in the spinal cord, including: glutamatergic, and GABAergic neurotransmissions (Allain et al., 2010; Hori et al., 1996; Thiriet et al., 1994). Moreover, 5-HT displays a complex modulatory role in pain processing, with both pro- and anti-nociceptive effects (Bardin et al., 1997; Viguier et al., 2013). Similarly, 5-HT can promote and inhibit motor function (Beato and Nistri, 1998; Kim et al., 1999; Perrier et al., 2017). Multiple factors may account for these contradictory roles of 5-HT including: activation of diverse 5-HT receptors; different brainstem origins of 5-HT projections; terminal locations of 5-HT axons; and the pathophysiological status of the spinal cord [for review see (Viguier et al., 2013)]. In addition, 5-HT-mediated modulation of pain processsing depends on the phenotype of target neurons that expresses 5-HT receptors (Viguier et al., 2013).

### 2.5. 5-HT modulation of motoneuron physiology

Multiple 5-HT receptors are expressed by motoneurons in the spinal cord. In general, 5-HT increases motoneuron excitability directly via activation of 5-HT receptors and consequent modulation of multiple ion channels [for reviews see (Perrier and Cotel, 2015; Perrier et al., 2013)]. Explicitly, 5-HT (1) activates $K^+$ and $Na^+$ channels that depolarizes motoneurons towards activation threshold, (2) inhibits $Ca^{2+}$ activated $K^+$ conductance, thereby increasing motoneuron firing frequency; and (3) induces persistent inward current, mediated by voltage-gated $Ca^{2+}$ and $Na^+$ channels, leading to continued motoneuron depolarization and amplified synaptic input (Perrier et al., 2013). Recent studies further suggest that 5-HT-mediated modulation of motoneuron excitability depends on the level of this neurotransmitter released, and on the specific location of 5-HT receptors in the spinal cord (Perrier and Cotel, 2015). Thus, low/moderate 5-HT release activates intrasynaptic 5-HT receptors that promotes $Ca^{2+}$ channel opening and increases motoneuron excitability, whereas high/intense 5-HT release diffuses away from synapses and activates extrasynaptic 5-HT receptors that inhibit $Na^+$ channels and block action potentials (Perrier and Cotel, 2015). 5-HT also modulates motoneuron excitability indirectly via its influence on spinal interneurons (Abbinanti and Harris-Warrick, 2012; Díaz-Ríos et al., 2007; Zhong et al., 2006). Indeed, 5-HT increases spinal interneuron excitability by promoting voltage-gated $Ca^{2+}$ channel activation (Abbinanti and Harris-Warrick, 2012).

### 2.6. 5-HT Regulation of the bladder function

Bladder function regulation is mediated via the autonomic (sympathetic and parasympathetic) and somatic nervous systems. The sympathetic pathway inhibits bladder detrusor contraction during low urine volume in the bladder, thereby promoting continence. Upon increased urine volume, and thus elevated intravesical pressure, the parasympathetic pathway triggers bladder detrusor contraction, thereby enabling urine void [for review see, (Fowler et al., 2008)]. In parallel, there is also a coordinated interaction between autonomic bladder and somatic sphincter muscle activity, regulated at cortical and subcortical levels that influences micturition (Griffiths et al., 2005).

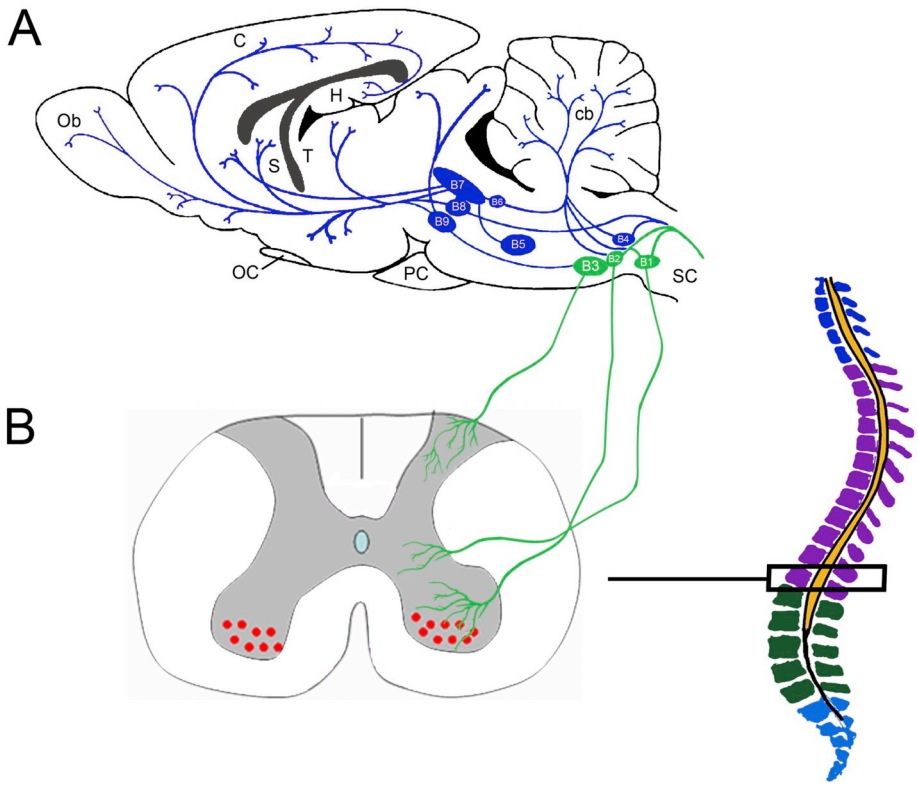


**Fig. 2.** Serotonin innervation of the central nervous system. Schematic diagram illustrating the major rostral (blue) and caudal (green) 5-HT projections (A). Schematic diagram demonstrating 5-HT innervation of the spinal cord (B). Note that the raphe magnus (B3) projects to the dorsal horn, the raphe pallidus (B1) projects to the ventral horn, and the raphe obscurus (B2) innervates the intermediate zone of the spinal cord. Red dots indicate motor neurons. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Initial anatomical studies in 1964 identified dense 5-HT axons innervating the sympathetic and parasympathetic nuclei throughout the spinal cord, suggesting 5-HT-mediated modulation of the autonomic nervous system (Dahlström and Fuxe, 1964). More recent anatomical tracing confirmed that caudal 5-HT projections, particularly from the raphe magnus (B3), are involved in regulating bladder activity (Ahn et al., 2018). 5-HT controls bladder function at multiple levels, including parasympathetic input to the bladder and somatic input to the external urinary sphincter (Berger et al., 2009). Early pharmacological studies suggested that central 5-HT neurotransmission inhibits micturition (De Groat and Ryall, 1967; Ryall and DeGroat, 1972), as 5-HT application to the spinal preganglionic neurons inhibited micturition by stimulating the sympathetic pathway (De Groat and Ryall, 1967) and depressed the parasympathetic pathway (Ryall and DeGroat, 1972). Electrical stimulation of the raphe magnus (B3) also inhibits micturition by blocking rhythmic bladder contractions (McMahon and Spillane, 1982; Sugaya et al., 1998). More recent data suggest that the 5-HT-mediated effect on bladder function depends on activation of distinct 5-HT receptors [for review see (Ramage, 2006)]. In addition, there are also reports of species-dependent differences in the effects of 5-HT on micturition. For instance, 5-$HT_{1A}$, 5-$HT_{2A/2C}$, 5-$HT_4$, and 5-$HT_7$ receptor activation in rats promotes micturition (Chen et al., 2013; Espey et al., 1992; Ishizuka et al., 2002; Read et al., 2003). Similarly, 5-$HT_1$ and 5-$HT_2$ receptor activation facilitates micturition in rabbits (Lychkova and Pavone, 2013). However, 5-$HT_{1A}$ receptor activation inhibits micturition in cats (Thor et al., 2002). Further studies using genetic targeting of 5-HT receptors are necessary to uncover the role of 5-HT in bladder function between different species.

## 3. Spinal cord injury induces differential rostro-caudal responses of 5-HT axons

SCI induces variable alterations in 5-HT projections, depending on the type of injury, lesion severity and animal model. Importantly, the response of 5-HT axons to SCI differs according to their location with respect to the injury site. Caudal to the lesion site, 5-HT axons degenerate and the degree of axonal degeneration positively correlates with lesion severity (Faden et al., 1988). In the rat spinal cord, 5-HT immunoreactivity is significantly reduced as compared to normal condition (Fig. 3A) immediately caudal to the lesion, but it is less affected further caudally 2 weeks after moderate thoracic (T9/T10) contusion [10 g dropped from 25 mm height] (Hayashi et al., 2010; Holmes et al., 2005) (Fig. 3B). Two weeks after severe T9/T10 contusion [10 g dropped from 50 mm height], 5-HT immunoreactivity is significantly decreased caudally throughout the remaining rat spinal cord (Hayashi et al., 2010) (Fig. 3C). T8 lateral hemisection of the rat spinal cord not only induces complete loss of 5-HT axons proximately caudal to the lesion but also causes a transient decrease in 5-HT axons ipsilateral to lesion that extends caudally to lumbo-sacral levels (Fig. 3D). Ipsilateral 5-HT axons then re-appear at lumbo-sacral levels within 3–4 weeks following injury (Saruhashi et al., 1996). Cervical (C4) lateral spinal cord hemisection in the rat also reduces ipsilateral 5-HT axons caudal to the lesion down to lumbar L2 – L4 levels without affecting contralateral 5-HT axons (Filli et al., 2011). However, and conversely to the Saruhashi study, 5-HT axons within the ipsilateral lumbar spinal cord remain low up to 4 weeks after injury. Although differences in anatomical level of injury may explain these contradictory findings, other factors, including strain, and sex differences are also important contributors. Complete section of the spinal cord in both rats (at T9/T10) and mice (at T8) result in permanent loss of 5-HT axons caudal to the lesion (Holmes et al., 2005; Lee et al., 2010b) (Fig. 3F).

In the mouse spinal cord, lumbar 5-HT axons remain intact 8 weeks after moderate T10 contusion [60 kilodynes, kdyn] (Xia et al., 2017). However, 8 days after T8 dorsal hemisection, which is more severe than in the Xia et al. study, 5-HT axons are rarely found caudal to the lesion (Camand et al., 2004). Importantly, after both contusion or dorsal hemisection, dorsal 5-HT projections undergo severe degeneration due to the dorsal location of the lesion whilst the ventral 5-HT projections are spared (Hayashi et al., 2010; Xia et al., 2017).

The response of 5-HT axons rostral and caudal to the site of SCI differs substantially in both rats and mice. Indeed, 5-HT projections immediately rostral to the lesion undergo pronounced sprouting in both

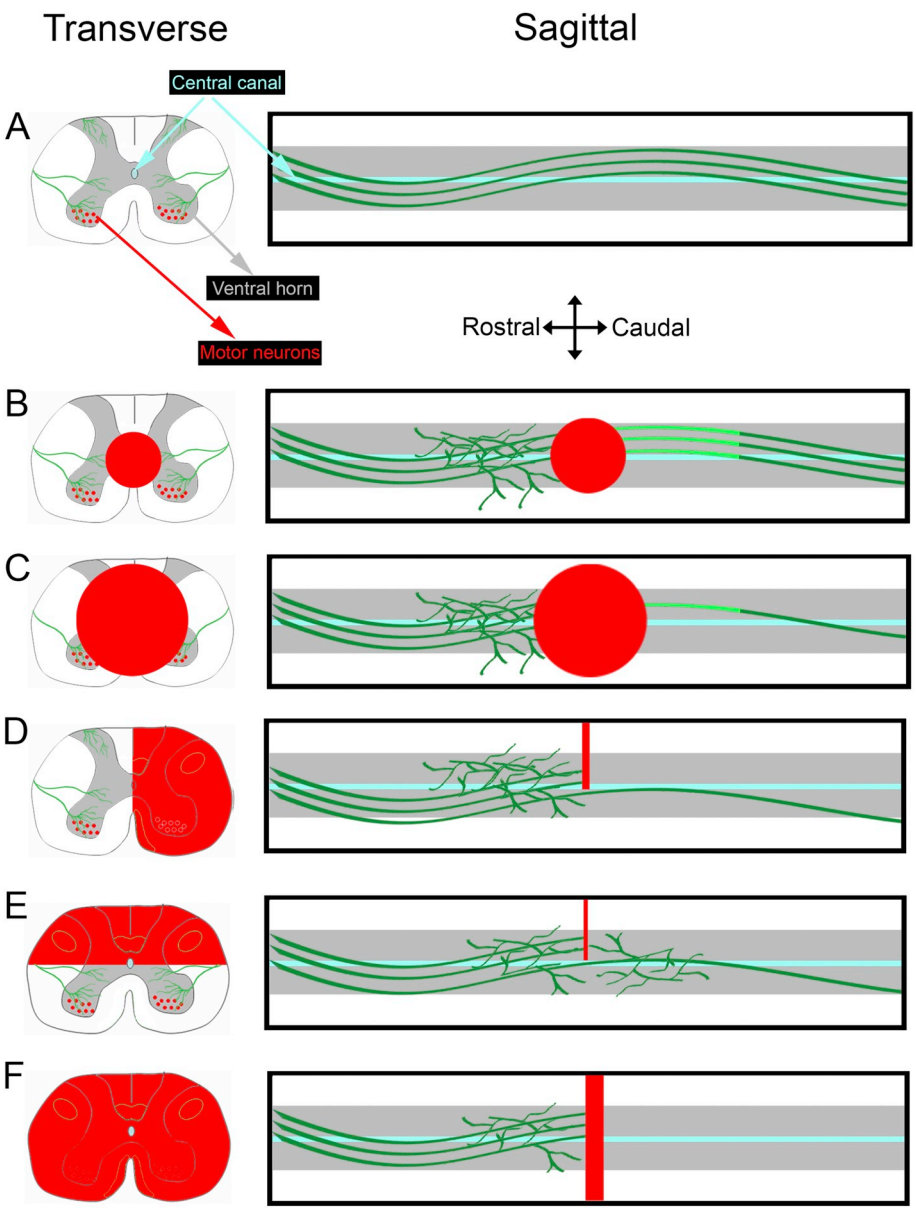


**Fig. 3.** Serotonin innervation of the spinal cord after different lesion severities. Schematic diagram showing 5-HT axons in the intact spinal cord (A) and their response after moderate contusion (B), severe contusion (C), lateral hemisection (D), dorsal hemisection (E), and complete section (F). Note the decrease in 5-HT innervation of the spinal cord caudal to the lesion site, that is more evident after severe SCI. Also note consistent 5-HT axon sprouting immediately rostral to the lesion site, irrespective of lesion severity.

species using several SCI models (Fig. 3).

Multiple studies have found an increase in 5-HT immunoreactivity rostral to the site of injury after spinal cord lesion. In rats, 5-HT immunoreactivity immediately rostral to the lesion site increases within 2 days after moderate T9/T10 contusion [10 g dropped from 25 mm height] and remains elevated up to 6 weeks post-injury (Holmes et al., 2005) (Fig. 3B). Likewise, increased 5-HT immunoreactivity proximately rostral to the lesion site is observed 2 weeks after severe T9/T10 contusion [10 g dropped from 50 mm height] (Hayashi et al., 2010) (Fig. 3C). Similarly, 5-HT immunoreactivity increases rostral to the lesion site 1 week after T8 lateral spinal cord hemisection (Saruhashi et al., 1996) (Fig. 3D). 5-HT content rostral to the lesion also continuously increases within 3–5 days following a complete section of the spinal cord at mid-thoracic level (Magnusson, 1973). In contrast to many reports discussed above, one study found stable 5-HT immunoreactivity at C6-C8 after C4/C5 lateral hemisection (Filli et al., 2011). As mentioned earlier, differences in anatomical level of the lesion, strain, and sex may explain these contradictory findings.

In keeping with the majority of studies, 8 days after T8 dorsal hemisection of the mouse spinal cord, 5-HT axons predominate rostrally to the lesion site, and increase in density within a month following injury (Camand et al., 2004). By 6-months after T8 dorsal hemisection, 5-HT axon density further increases rostro-caudal to the lesion site, though they do not enter the lesion core (Camand et al., 2004) (Fig. 3E). Increased 5-HT immunoreactivity rostral to the lesion site is also reported 8 weeks after a T9 complete crush of the mouse spinal cord (Inman and Steward, 2003).

Altogether, studies using various rodent models of SCI have consistently shown that 5-HT axons caudal to the lesion epicenter degenerate whereas immediately rostral to the lesion site there is an increase in 5-HT axon density, irrespective of time after injury and lesion severity. Given the widespread distribution of 5-HT axons throughout the spinal cord, incomplete injury models, where some 5-HT axons are spared, makes it impossible to discriminate between 5-HT axon

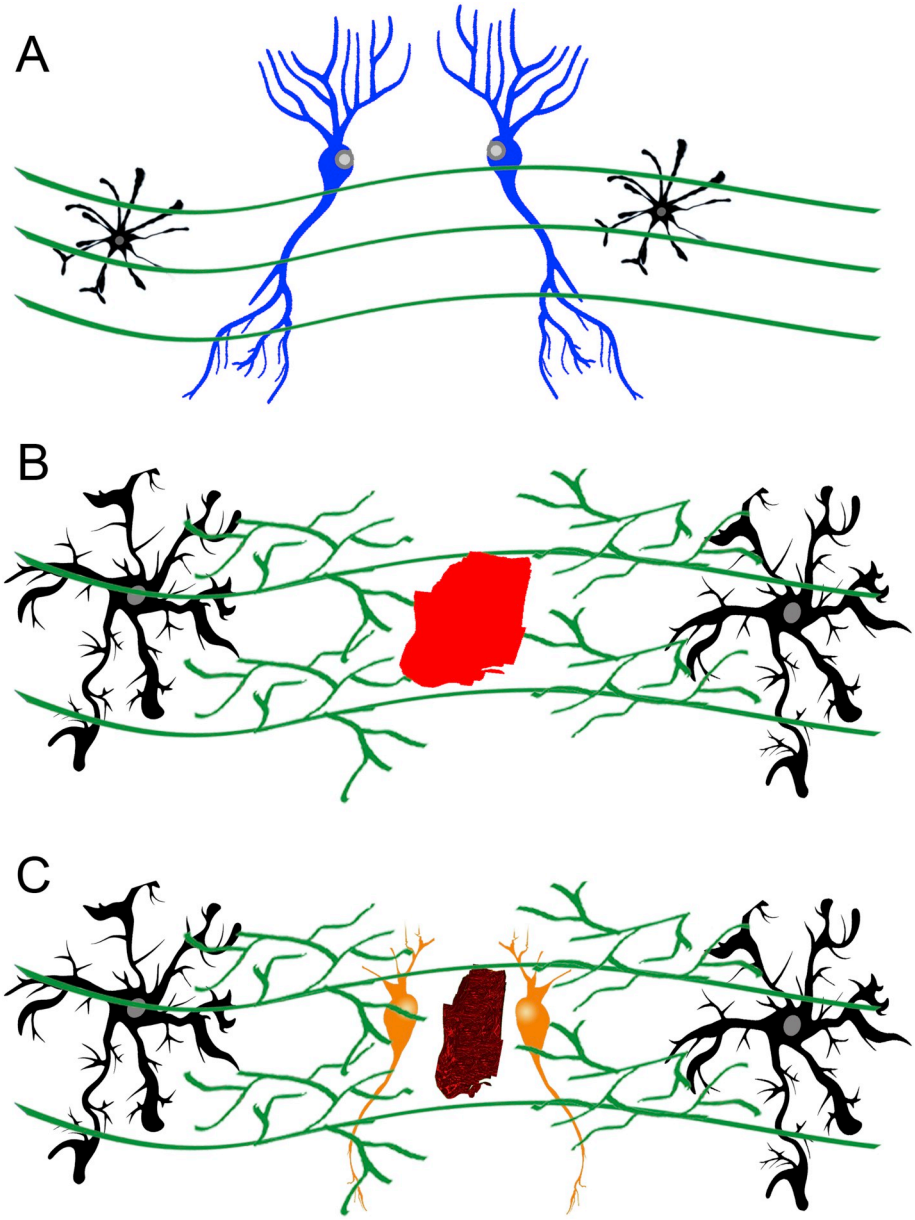


**Fig. 4.** Serotonin axon sprouting after CNS insult and neurodegenerative diseases. Schematic diagram illustrating 5-HT axons in the intact CNS (A) and their sprouting after neurotoxin injection (B), and β-amyloid (Aβ) deposition (C). Note that 5-HT axon sprouting is accompanied by a pronounced increase in astrocyte reactivity (astrogliosis).

sprouting (re-growth from spared axons) or regeneration (re-growth from injured axons either directly from the severed tip or proximal to it) [definitions from (Tuszynski and Steward, 2012)]. However, the increase in 5-HT axon density immediately rostral to the lesion following complete crush of the spinal cord, where all 5-HT axons are lesioned (Inman and Steward, 2003), support axon regeneration. This suggests that 5-HT system plasticity results in specific regenerative abilities that allow active axon sprouting and regeneration after SCI. Currently, it is unclear whether 5-HT axon regeneration immediately rostral to the lesion occurs from injured tip or proximal to it. Interestingly, 5-HT axon sprouting and regeneration also occurs after a broad array of CNS insults and in a mouse model of neurodegenerative disease, as discussed below.

## 4. Potential mechanisms underlying 5-HT axon sprouting and regeneration after CNS insult and in neurodegenerative disease

Acute brain injury in rats induced by several neurotoxins, including: ibotenic acid (Harkany et al., 2000; Zhou et al., 1995), NMDA (Harkany et al., 2000), and β-amyloid (Aβ) (Harkany et al., 2001) triggers strong 5-HT axonal sprouting (Fig. 4A&B). Initially, NMDA and Aβ elicit cholinergic neuronal loos and 5-HT axon degeneration characterized by beaded appearance with swollen spherical or oval varicosities, within the injection site (Harkany et al., 2000; Harkany et al., 2001). Subsequently, NMDA and Aβ trigger 5-HT axon sprouting and regeneration, though it is currently unclear whether regeneration occurs from their injured tips, proximal, or distal segments. Likewise, 5-HT axons distal to the injury site degenerates after different traumatic brain injuries, such as persistent open skull condition and controlled cortical impact (Kajstura et al., 2018). By 1 month post-lesion, 5-HT axon density increases distal to the injury site (Kajstura et al., 2018), although the

authors do not discriminate between axon sprouting and regeneration. Moreover, after neocortical stab injury in mice, 5-HT axons re-grow from their cut ends and traverse the stab rift (Jin et al., 2016), confirming spontaneous regeneration. P-chloroamphetamine (PCA) is an amphetamine derivative that selectively damages 5-HT axons (Mamounas and Molliver, 1988; Mamounas et al., 1991), however, 5-HT axons gradually re-appear within 2–8 months following PCA treatment in rat neocortex, suggesting axon sprouting and regeneration (Molliver et al., 1990). A recent *in vivo* imaging study confirms 5-HT axon degeneration and subsequent spontaneous regeneration following systemic PCA treatment in mice (Jin et al., 2016). 5-HT axons persist within the lesion edge and afterward sprout following thermo-coagulatory lesion in the rat frontoparietal cortex (Hawthorne et al., 2011). We also found serotonergic axon sprouting and an increase in serotonergic terminals, concurrent with Aβ deposition, in a transgenic mouse model of Alzheimer's disease (AD) (Noristani et al., 2011; Noristani et al., 2010) (Fig. 4A&C), without serotonergic axon degeneration throughout the disease progression. Alike, ibotenic acid injection in the rat striatum and hippocampus also causes serotonergic axon sprouting without prior degeneration (Noristani et al., 2010; Zhou et al., 1995), suggesting outgrowth from undamaged axons.

An important question concerns the intrinsic machinery that enables 5-HT axons not only to survive within an inhibitory environment (with or without initial degeneration) but also to undergo active sprouting and regeneration after different CNS insults and neurodegenerative diseases. One possibility includes an increased expression of growth-promoting factors. Indeed, the high expression level of growth-associated-protein-43 (GAP43) and β1 integrin by 5-HT neurons may support their longer growth in the presence of growth-promoting laminin (Hawthorne et al., 2011). Moreover, 5-HT axons simultaneously display reduced inhibition in the presence of the axon growth-inhibitory factors chondroitin sulfate proteoglycans (CSPGs) (Hawthorne et al., 2011), suggesting reduced expression of CSPG receptors. A more recent study suggests that 5-HT axons express high levels of proteases that degrade CSPGs, which may support their survival and sprouting within the injury environment (Tran et al., 2018). In contrast to CSPGs, 5-HT axon sprouting following SCI is influenced by the presence of myelin-derived inhibitors, including: Nogo, oligodendrocyte-myelin glycoprotein (OMgp), and myelin-associated glycoprotein (MAG) (Kim et al., 2004; Li and Strittmatter, 2003; Wang et al., 2011b). All three of these anti-regenerative proteins bind to a Nogo-66 receptor (NgR) that is expressed, among others, by 5-HT neurons, and may prevent their sprouting and possible regeneration after SCI (Kim et al., 2004). In keeping with this, pharmacological inhibition of NgR promotes sprouting of 5-HT axons after T6 dorsal hemisection in mice (Li and Strittmatter, 2003) or moderate T7 contusion [10 g dropped from 25 mm height] in rats (Wang et al., 2011b). In mice, pan-neuronal overexpression of an endogenous NgR antagonist also triggers 5-HT axon sprouting caudal to the lesion site following T8 dorsal hemisection of the spinal cord (Hirokawa et al., 2017). Also, in mice, an earlier report found that genetic deletion of NgR enables regeneration of 5-HT axons across the lesion site following T8 complete section of the spinal cord (Kim et al., 2004). However, a subsequent study showed that genetic deletion of myelin inhibitors does not promote 5-HT axon regeneration after T8 complete section of the spinal cord (Lee et al., 2010b), suggesting that further studies are needed to clarify the interaction between myelin inhibitors and 5-HT axons after SCI.

Another possibility that may underlie 5-HT axon sprouting and regeneration after CNS injury is their lack of synaptic contacts. As mentioned earlier, 5-HT neurotransmission predominantly occurs via volumetric transmission that involves 5-HT diffusion across the extracellular space prior to reaching its high affinity receptor targets (Miner et al., 2000; Oleskevich et al., 1991; Séguéla et al., 1989). Volumetric transmission can be achieved even when regenerating axons innervate a region proximal to their former location (Jin et al., 2016). In addition, accumulating evidence suggests that synaptic contacts stabilize axons and participate in their regeneration failure within the CNS [for a review see (Meves and Zheng, 2016)]. In line with this, the presence of an intact synaptic branch prevents axon regeneration in *Caenorhabditis elegans* (*C. elegans*) following laser ablation (Wu et al., 2007). In addition, *in vivo* sensory axon regeneration in the mouse spinal cord depends on ablation of both ascending and descending branches, suggesting that a remaining intact branch with presumed synaptic contact suppresses axonal re-growth (Chen et al., 2011). Indeed, establishment of synaptic-like contacts between regenerating sensory axons and non-neuronal cells prevents their further growth within the spinal cord in mice (Di Maio et al., 2011) and rats (Filous et al., 2014). Although precise molecular mechanisms underlying synapse-mediated regeneration failure is currently unknown, a recent study suggests the involvement of the alpha2delta2 accessory subunit of voltage-gated $Ca^{2+}$ channels (Tedeschi et al., 2016).

Taken together, these data suggest that 5-HT axon sprouting and regeneration occurs after multiple CNS insults. Available data on the interaction between 5-HT axons and myelin-derived inhibitors are currently inconclusive; however, growing evidence suggests that the lack of synaptic contact may be a key component that enables sprouting and regeneration of 5-HT axons after CNS insults and neurodegenerative diseases.

## 5. 5-HT-based therapeutic strategies

### 5.1. Chronic elevation of 5-HT neurotransmission promotes 5-HT axon sprouting in the intact brain

Increased 5-HT precursor (TrP) intake is a viable non-pharmacological approach used to elevate 5-HT neurotransmission in the CNS (Haider et al., 2007; van der Stelt et al., 2004). A chronic (1 month) increase in dietary TrP intake stimulates sprouting of serotonergic axons in the adult mouse hippocampus (Noristani et al., 2012). Blocking 5-HT re-uptake also increases 5-HT neurotransmission and is routinely used as anti-depressive medication (Tao et al., 2000); chronic (4–14 weeks) antidepressant treatment in rats promotes 5-HT axon sprouting in multiple brain regions, including: the cortex, the forebrain, the lateral septal nucleus, and the nucleus accumbens (Horne et al., 2008; Zhou et al., 2006). Of note, this 5-HT neurotransmission-mediated increase in axonal sprouting is due to outgrowth from undamaged axons in the intact brains, and the mechanism is therefore likely independent of the lesion-induced 5-HT axon sprouting and regeneration discussed earlier. Indirect evidence suggests that a chronic increase in 5-HT neurotransmission following injury may also promote sprouting of 5-HT axons. Indeed, intermittent electrical stimulation of the raphe magnus (B3) between 1 and 7 days following T8 mild/moderate contusion [10 g dropped from 12.5 mm] increases 5-HT terminals rostrocaudal to the lesion site in rats (Hentall and Gonzalez, 2012). While the underlying mechanisms responsible for neurotransmitter-mediated increased 5-HT axonal sprouting awaits further study, one possibility is an upsurge in neuronal activity, as shown for corticospinal axons (Carmel et al., 2010; Martin, 2016; Song et al., 2016). Cell-specific transcriptomic and proteomic studies would certainly help to uncover molecular mechanisms underlying increased neurotransmitter mediated 5-HT axon sprouting. Regardless of the causal molecular mechanisms, several studies have investigated the effect of elevated 5-HT neurotransmission on functional recovery after SCI, and these are discussed below.

### 5.2. Increased 5-HT neurotransmission and its effect on functional recovery after SCI

Evidence relating to the positive role of elevated 5-HT neurotransmission on functional recovery after SCI has been predominantly gained from rat injury models, in which the 5-HT precursor 5-hydroxytryptophan (5-HTP) or 5-HT itself were targeted, either individually

or in combination with other drugs.

In rats, at a physiological level, acute 5-HTP administration induces electrical discharges in hindlimb muscles 20 days after T5 transection (Barbeau et al., 1981) and causes tonic activity in phrenic motoneurons following C1/C2 spinal cord transection (Mitchell et al., 1992). At a functional level, acute 5-HTP administration facilitates flexor reflex and triggers spontaneous movement 2 months after T4 – T6 spinal cord transection in rats (Nozaki et al., 1977). Similarly, in T12 transected cats, acute 5-HTP treatment immediately before behavioral testing improves several parameters of locomotion, including step length, hindlimb extensor, flexor, and axial muscle amplitudes (Barbeau and Rossignol, 1990). Furthermore, acute intrathecal 5-HT administration attenuates mechanical allodynia and thermal hyperalgesia 28 days after T13 lateral hemisection of the spinal cord in rats (Hains et al., 2002). A combinatorial approach to elevate 5-HT neurotransmission seems to be more effective in improving functional recovery following SCI. For instance, acute 5-HTP administration combined with carbidopa (which inhibits peripheral 5-HTP degradation and promotes its CNS delivery), increases hindlimb motor function in rats 9 weeks after both moderate and severe T9/T10 spinal cord contusions [10 g dropped from 25 and 50 mm, respectively) (Hayashi et al., 2010). Likewise, acute 5-HTP treatment associated with pargyline (which prevents 5-HT degradation) restores respiratory-related activity of the phrenic nerve ipsilateral to the lesion after C2 lateral hemisection of the spinal cord in rats (Zhou and Goshgarian, 2000). Also, intrathecal co-administration of 5-HT and the antidepressant fluvoxamine further improves recovery of pain behavior following T13 lateral hemisection of the rat spinal cord (Hains et al., 2002). It is important to note that acute 5-HTP or 5-HT treatment either alone or in combination with other drugs does not induce changes in 5-HT axon sprouting or regeneration after SCI (Azam et al., 2015; Hayashi et al., 2010). Repeated 5-HTP administration, causing a chronic increase in 5-HT neurotransmission, may thus be necessary to promote 5-HT axon sprouting or regeneration in the injured spinal cord. In line with this, 5-HT terminals increase rostro-caudal to the lesion site only after chronic (1 week) raphe magnus (B3) electrical stimulation but not after 1–3.5 days of 5-HTP administration (Hentall and Gonzalez, 2012). Similarly, chronic (9 weeks) treatment with the antidepressant fluoxetine is necessary to trigger corticospinal axon sprouting in rats after C4 dorsal funiculus crush, although the authors did not examine changes in 5-HT axons (Scali et al., 2013). Prolonged (2 weeks) daily electrical stimulation of the raphe magnus (B3) after mild/moderate T8 contusion [10 g dropped from 12.5 mm] in rats further improves open-field motor performance, compared to 1 week of daily stimulation (Hentall and Gonzalez, 2012). Equally, chronic (1–3 weeks) intrathecal 5-HT injection coupled with tail pinching are needed to improve several parameters of locomotion in T8/T9 transected rats, including: longer locomotor sequences with a larger number of successive steps, better bodyweight support, improved interlimb coordination, and increased electromyographic burst amplitude (Feraboli-Lohnherr et al., 1999).

In mice, daily (7 days) treatment with fluoxetine reduces inhibitory interneuron expression in the medial prefrontal cortex and prolongs the recovery window after stroke (Ng et al., 2015), suggesting modulation of excitatory/inhibitory balance. Intact rats chronically (3 weeks) treated with fluoxetine also display reduced inhibition and increased excitation (measured by analyzing the expression levels of the vesicular transporter proteins for glutamate and GABA) within the motor cortex and the spinal cord (Scali et al., 2013). These findings suggest that elevated 5-HT neurotransmission via fluoxetine treatment modulates excitatory/inhibitory balance that in turn extends the recovery window after stroke (Ng et al., 2015). Whether chronic increase in 5-HT neurotransmission also affects excitatory/inhibitory imbalance following SCI awaits further studies. Other pharmacological strategies aimed at boosting the 5-HT system include administration of specific 5-HT receptor agonists/antagonists. However, a comprehensive analysis of 5-HT receptor targeting after SCI is beyond the scope of the current review [for a recent review see (Zhang, 2016)].

In sum, these studies suggest that elevated 5-HT neurotransmission improves functional recovery following SCI. A combinatorial approach, targeting multiple aspects of the 5-HT system, particularly when chronically applied, appears to be more potent in recovering motor function after spinal cord lesion. In the following section, we will focus on studies that applied embryonic raphe transplantations and their effect on anatomical and functional recovery following SCI.

### 5.3. Intraspinal transplantation of the raphe nuclei after SCI

Another strategy to boost the 5-HT system following lesion is intraspinal transplantation. The first attempt to repair the injured spinal cord using neural cell transplantation took place in Canada over a century ago when a Scottish physician, David Alexander Shirres (1905), implanted a spinal cord segment from a dog into the spinal cord of an adult SCI patient, without success (Robb, 1991; Shirres, 1905). Following the important discovery by Samuel David and Alberto J. Aguayo, demonstrating that peripheral nerve transplantation into the injured rat spinal cord can act as a “bridge” for CNS axon regeneration (David and Aguayo, 1981), numerous attempts have been made to transplant different neural populations as therapeutic approaches to improve functional recovery after SCI.

The rodent spinal cord generates locomotor rhythmic activity through a specific neuronal network called the central pattern generator (CPG) for locomotion that is directly modulated by descending 5-HT projections (Cazalets et al., 1992; Gimenez y Ribotta et al., 1998a). Given the critical role of 5-HT neurotransmission in modulating motor function and the increase in 5-HT axonal plasticity after CNS insults, embryonic raphe nuclei are a particularly attractive source for transplantation studies aiming at providing a continuous supply of 5-HT caudal to the lesion.

At the anatomical level, transplanted embryonic raphe-derived 5-HT neurons grow to their normal size (Sławińska et al., 2000). Within 30 days post-transplantation, 5-HT axons extend from their soma and subsequently outspread up to 20 mm between 60 and 90 days post-transplantation (Nygren et al., 1977; Privat et al., 1988; Privat et al., 1986; Privat et al., 1989). Transplant-derived 5-HT axons innervate their main target areas in the injured spinal cord in a way that closely resembles intact animals (Privat et al., 1986; Ribotta et al., 2000). In particular, transplantation of B1, B2, and B3 raphe nuclei reinnervate their specific targets, namely the ventral horn (B1), the intermediolateral column (B2), and the dorsal horn (B3) (Rajaofetra et al., 1992). At both light and electron microscopic levels, transplant-derived 5-HT neurons survive and integrate within the injured host spinal cord up to 1-year post-transplantation in rats (Privat et al., 1988; Privat et al., 1989). Likewise, raphe suspension from fetal *Macaca fascicularis* transplanted 1 week after T6 transection in adult primate also survives at least up to a month post-transplantation (Rajaofetra et al., 1989).

At a functional level, intraspinal embryonic raphe transplantation is effective in improving motor function even when transplanted 1 week (see Table 1) or 1 month post-SCI (Majczyński et al., 2005; Sławińska et al., 2000; Sławińska et al., 2013). 5-HT released from such transplanted raphe grafts is necessary for functional improvement, since increasing 5-HT neurotransmission, by blocking its re-uptake, further facilitates locomotor recovery (Feraboli-Lohnherr et al., 1997). Greater improvement in functional recovery is achieved when only the descending (B1, B2&B3) raphe nuclei are transplanted into the injured spinal cord (Sławińska et al., 2013). This may be because 5-HT neurons within descending nuclei are programmed to innervate the spinal cord (Foster et al., 1989). Apart from one exception [e.g. (Rajaofetra et al., 1989)], embryonic raphe transplantation studies have predominantly used rat models of SCI (Table 1). It is also important to note that raphe transplants do not exclusively contain 5-HT neurons. In fact, 5-HT neurons account for only 2–4% of the embryonic raphe suspension (Privat et al., 1988), although they might be more resistant than other neurons within the injured milieu. Indeed, raphe transplants express

**Table 1**
Intraspinal transplantation of embryonic raphe after SCI.

| Strain, species (Gender) | SCI model | Transplant approach | Time (Post-SCI) | Anatomical finding (Time post-transplantation) | Functional recovery (Time post-transplantation) | Reference |
|---|---|---|---|---|---|---|
| Adult sprague-dawley rats (males and females) | Lumbar transection | E17–19 raphe suspension | Immediately before SCI | 5-HT axons extension from their soma (30 days)<br>5-HT axons extension over 10 mm below the lesion (90 days) | NA | (Nygren et al., 1977) |
| Adult sprague-dawley rats (males) | Low thoracic transection | E14 raphe suspension | 7 days | Targeted 5-HT innervations caudal to the lesion similar to intact animals (10–60 days)<br>5-HT axons extend over 20 mm caudal to the lesion (60 days) | NA | (Privat et al., 1986) |
| Adult sprague-dawley rats (males) | T7/8 transection | E13–14 raphe suspension | 7 days | Readily apparent 5-HT axons caudal to the lesion (10 days)<br>5-HT axons extend over 20 mm caudal to the lesion (60 days)<br>Sustained 5-HT axons caudal to the lesion (1 year) | NA | (Privat et al., 1988) |
| Adult sprague-dawley rats (males) | T7/8 Transection | E13–14 raphe suspension | 7 days | 5-HT axon extension (10 days)<br>5-HT axons extend over 20 mm caudal to the lesion (60 days-1 year)<br>Axosomatic and axodendritic synapses within the anterior horn and intermediolateral column, similar to un-injured control (60 days-1 year) | Restored ejaculation reflexes (2 months) | (Privat et al., 1989) |
| Adult rats (NS) | 5,7-dihydroxytryptamine lesion of central 5-HT or T8 transection | E raphe from rostral and caudal nuclei | 10–14 days | 5-HT axons re-innervation of the spinal cord making contact with residual motoneurons (1 year)<br>Reduced cell survival of rostral (4%) than caudal (25%) raphe nuclei (1 year) | NA | (Foster et al., 1989) |
| Adult sprague-dawley rats (males) | T7 transection | E14 B1-B2 or B3 raphe nuclei | 7 days | 5-HT innervations of the ventral horn and the intermediolateral cell column by B1-B2 grafts (3 months)<br>5-HT innervation of the dorsal horn by B3 graft (3 months) | NA | (Rajaofetra et al., 1992) |
| Adult sprague-dawley rats (females) | T8/9 transection | E14 raphe suspension | 8 days | 5-HT reinnervations caudal to the lesion making synaptic contacts with host interneurons and/or motoneurons (1–3 months) | Increased excitability of the spinal stepping generator, prolonged locomotor episodes and shortened step cycles (1–3 months) | (Yakovleff et al., 1995) |
| Adult sprague-dawley rats (females) | T8/9 transection | E14 raphe<br>With or without intrathecal administration of noradrenergic specific neurotoxin (6-hydroxydopamine) 2 months post-transplantation | 8 days | 5-HT re-innervation of the lumbar enlargement (6 months) | Recovery of hindlimb bilateral, alternating, rhythmic locomotor-like activity (6 months)<br>No effect on improved functional recovery upon elimination of noradrenergic input<br>Facilitation of locomotor-like activity upon 5-HT re-uptake blockade by zimelidine. | (Feraboli-Lohnherr et al., 1997) |
| Adult sprague-dawley rats (females) | T8 transection | E14 raphe suspension | 7 days | 5-HT positive neurons and axons caudal to the lesion (2 months)<br>5-HT axon extension caudal to L2 (2 months) | Recovery of standing up, supporting hindquarter weight, and walking on a treadmill with their four limbs upon tail pinching (2 months) | (Gimenez y Ribotta et al., 1998b) |
| Adult sprague-dawley rats (females) | T8 transection | E14 raphe suspension | 7 days | 5-HT reinnervation of the L1-L2 levels (2 months) | Normal locomotor pattern (2 months) | (Ribotta et al., 2000) |
| Adult wistar rats (NS) | T9/T10 transection | A piece of E14-E15 solid raphe tissue containing B1 and B2 | 1 month | 5-HT axon extension 15 mm caudal to the lesion (2 months) | Better hindlimb motor function recovery and improved interlimb co-ordination (2 months) accompanied by a relatively high EMG amplitude (2 months) | (Sławińska et al., 2000) |

(*continued on next page*)

**Table 1** (*continued*)

| Strain, species (Gender) | SCI model | Transplant approach | Time (Post-SCI) | Anatomical finding (Time post-transplantation) | Functional recovery (Time post-transplantation) | Reference |
|---|---|---|---|---|---|---|
| 30 days old sprague-dawley rats (males) | T13 lateral hemisection | E13 raphe derived RN46A-B14 cells bioengineer-red to secrete both 5-HT and BDNF | 28 days | Increase in 5-HT and BDNF in CSF (28 days) | Improved BBB function (2–6 weeks)<br>Reduced fore- and hindlimbs mechanical allodynia and thermal hyperalgesia (2–6 weeks) | (Hains et al., 2001) |
| Adult wistar rats (NS) | T9 transection | A piece of E14-E15 solid raphe tissue containing B1 and B2 | 1 month | NA | Regular alternating hindlimbs movements on a treadmill with ground plantar contact during the stance phase, and ankle dorsiflexion during the swing phase of each step cycle (2 months) | (Majczyński et al., 2005) |
| Adult wistar albino glaxo rats (females) | T9/T10 transection | A piece of E14 solid raphe tissue containing B1, B2 and B3 | 1 month | 5-HT axon extension caudal to the lesion up to L3 (2 months) | Full recovery of inter- and intralimb coordination upon tail stimulation on a treadmill (2 months) | (Sławińska et al., 2013) |

Key: Not stated (NS), Not assessed (NA), embryonic (E), Brain derived neurotrophic factor (BDNF), Cerebrospinal fluid (CSF), Electromyographic (EMG), Basso, Beattie and Bresnahan (BBB) locomotor scale.

noradrenergic markers, confirming that the raphe suspension is a mixed cell population (Gimenez y Ribotta et al., 1998a). Nevertheless, elimination of noradrenergic input in raphe-transplanted animals, using noradrenergic-specific neurotoxin (6-hydroxydopamine), has no effect on improved functional recovery (Feraboli-Lohnherr et al., 1997). These findings suggest that 5-HT re-innervation of the transected spinal cord, in the absence of noradrenergic input, improves the hindlimb rhythmic motor activity that is characterized as locomotion. However, the functional consequence of exclusively transplanting embryonic 5-HT neuron after SCI remains unclear.

Overall, these studies suggest that transplanted raphe-derived 5-HT neurons integrate well into the injured spinal cord, re-innervate their original target regions, and improve functional outcome after SCI in rats. However, given the recently identified molecular and functional diversity between 5-HT synthesizing neurons (Okaty et al., 2015), it is necessary to uncover whether there is a specific sub-population of 5-HT neurons that are more resistant to demise following SCI. Additional studies are also necessary to reveal the molecular mechanisms underlying neurotransmission between the host and transplant-derived 5-HT neurons.

### *5.4. Therapeutic approaches promoting 5-HT axon sprouting and regeneration after SCI*

Therapeutic approaches in pre-clinical models of SCI include, among others: (1) reducing extrinsic inhibitors of regeneration such as gliosis, myelin inhibitors, and CSPGs; (2) promoting a pro-regenerative environment by transplantation of neural precursors (NPs), mesenchymal cells (MCs) or Schwann cells (SCs); (3) modulating neuroinflammation; and (4) increasing the pro-regenerative capacity of injured neurons. In all of these strategies, increased density of 5-HT axons caudal to the lesion site is a good indication of improved functional recovery (Table 2). While majority of these studies used incomplete lesions, there are also reports of increased 5-HT axon density caudal to the spinal cord lesion after complete transection (Hou et al., 2013; Kim et al., 2004), and complete compression (Papastefanaki et al., 2007), confirming both axon sprouting and regeneration.

Glial fibrillary acidic protein (GFAP) and vimentin (VIM) double knockout mice display reduced astrogliosis and 5-HT axon sprouting caudal to the lesion associated with improved motor recovery following T12 lateral hemisection of the spinal cord (Menet et al., 2003). Similarly, other transgenic mice with impaired NgR function also show 5-HT axon sprouting caudal to the lesion after T6/T7 dorsal over-hemisection (Li et al., 2005; Wang et al., 2011b).

Although intrathecal targeting of gliosis (Desclaux et al., 2015), myelin inhibitors (Bregman et al., 1995; Cao et al., 2008; GrandPré et al., 2002), or CSPGs (Alilain et al., 2011; Barritt et al., 2006; Lee et al., 2010a) promotes 5-HT axon sprouting immediately after injury, it raise significant limitation for translation to the clinic and do not benefit chronically injured patients, who account for most SCI cases (Furlan et al., 2013). Notably, delayed intrathecal targeting of glisosis (Hara et al., 2017), myelin inhibitors (Li et al., 2004; Wang et al., 2011b), or CSPGs (Wang et al., 2011a; Warren et al., 2018) also stimulates 5-HT axon sprouting associated with improved fucntional recovery. Importantly, delayed systemic treatment, focusing on extrinsic inhibitors or intrinsic pro-regenerative pathways, that induces improved functional recovery after SCI in both rats and mice also promotes 5-HT axonal sprouting caudal to the lesion site (Lang et al., 2015; Li and Strittmatter, 2003; Oatway et al., 2005; Ohtake et al., 2014).

Similarly, transplantation of NPs, MCs or SCs immediately after lesion (Papastefanaki et al., 2007), 7 days (Ghosh et al., 2012; Hodgetts et al., 2013; Perrin et al., 2010) or 14 days (Boido et al., 2009; Hou et al., 2013) post-lesion promotes sprouting and regeneration of 5-HT axons associated with functional improvement after SCI in rodents (see Table 2). These findings highlight that 5-HT axon sprouting and regeneration does not depend on immediate local therapy and may be

**Table 2**
Examples of preclinical approaches promoting 5-HT axon sprouting and regeneration after SCI.

| Strain, species (gender) | SCI model | Approach | Time (pre or post-SCI) | Anatomical finding (time post-SCI) | Functional recovery (time post-SCI) | Reference |
|---|---|---|---|---|---|---|
| Blocking extrinsic inhibitors such as gliosis | | | | | | |
| Adult mixed background mice (females) | T12 lateral hemisection | Global GFAP/VIM double knockout | From birth | Increased 5-HT axon sprouting ipsilateral to the lesion site (35 days) | Improved fine motor control and reduced footfall using grid runway test (28 days) | (Menet et al., 2003) |
| Adult C57BL/6 J mice (females) | T12 lateral hemisection | Intrathecal shGFAP and shVIM injection rostral, caudal and contralateral to the lesion | Immediately post- injury | Increased 5-HT axon sprouting caudal to the lesion site (14 and 35 days) | Improved fine motor control and reduced footfall using grid runway test (28 and 35 days) | (Desclaux et al., 2015) |
| Adult C57BL/6 J mice (females) | T10 severe contusion (60, kdyn) | Intrathecal injection of integrin β1 antibody in the lesion site | 9, 11, and 13 days post-injury | Increased 5-HT axon sprouting caudal to the lesion site (42 days) | Improved BMS score and grip walk parameters (stride length, stride width, paw rotation and total grip, 42 days) | (Hara et al., 2017) |
| Adult wistar rats (males) | T12 moderate compression | Intravenous administration of anti CD11d antibody via the tail vein | 2 h, 1, and 2 days post-injury | Increased 5-HT axon sprouting caudal to the lesion site (28 days) | Improved mechanical allodynia and BBB score (8–26 days) | (Oatway et al., 2005) |
| Blocking extrinsic inhibitors such as myelin inhibitors | | | | | | |
| Adult lewis rats (NS) | Midthoracic over hemisection | Intrathecal injection of NgR antibody | Immediately post- injury | Increased 5-HT axon sprouting caudal to the lesion site (84 days) | Improved motor function including contact placing response of the hind paws and increased stride length (60–90 days) | (Bregman et al., 1995) |
| Adult sprague-dawley rats (females) | T6 dorsal hemisection | Intrathecal injection of NgR antagonist peptide | Immediately post- injury | Increased 5-HT axon sprouting caudal to the lesion site (28 days) | Improved BBB score (7–28 days) | (GrandPré et al., 2002) |
| Adult C57BL/6 J mice (females) | T6/T7 dorsal over-hemisection | Subcutaneous administration of NgR antagonist peptide | 7 days post-injury | Increased 5-HT axon sprouting caudal to the lesion site (42 days) | Improved BMS score and reduced footfall using grid runway test (21–42 days) | (Li and Strittmatter, 2003) |
| Adult C57BL/6 J mice (females) | T6 dorsal hemisection or T8 transection | Global NgR knockout | From birth | T6 dorsal hemisection: increased 5-HT axon sprouting caudal to the lesion site (21 days)<br>T8 transection: increased 5-HT axon regeneration caudal to the lesion site (42 days) | Dorsal hemisection: improved BMS score (14–21 days)<br>Transection: improved weight-bearing posture (14–42 days) | (Kim et al., 2004) |
| Adult sprague-dawley rats (females) | T6/T7 dorsal over-hemisection | Intrathecal injection of soluble function-blocking NgR protein | Continuously post-injury | Increased 5-HT axon sprouting caudal to the lesion site (28 days) | Improved BBB score and reduced footfall using grid runway test (14–28 days) | (Li et al., 2004) |
| Adult C57BL/6 J mice (females) | T6/T7 dorsal over-hemisection | Transgenic over-expression of soluble function blocking NgR fragment | From birth | Increased 5-HT axon sprouting caudal to the lesion site (28 days) | Improved BMS score and reduced footfall using grid runway test (7–28 days) | (Li et al., 2005) |
| Adult sprague-dawley rats (females) | C4 lateral hemisection | Intrathecal injection of NgR antagonist peptide | Immediately post- injury | Increased 5-HT axon sprouting within the lesion site (28 days) | Improved forelimb function using CatWalk including coordination, stride length, | (Cao et al., 2008) |
| Adult C57BL/6 J mice (females) | T8 transection | Global NgR knockout | From birth | No 5-HT axon regeneration | NA | (Lee et al., 2010b) |
| Adult C57BL/6 J mice (females) | T6/T7 dorsal hemisection | Conditional NgR knockout | 56 days post-injury | Increased 5-HT axon sprouting caudal to the lesion site (210 days) | Improved BMS score (150–210 days) | (Wang et al., 2011b) |
| Adult sprague-dawley rats (females) | T7 moderate contusion (10 g dropped from 25 mm height) | Intrathecal injection of soluble function-blocking NgR protein | 90 days post-injury | Increased 5-HT axon sprouting caudal to the lesion site (180 days) | Improved BBB score and reduced footfall using grid runway test (110–180 days) | (Wang et al., 2011b) |
| Adult C57BL/6 J mice (males) | T8 dorsal hemisection | Pan-neuronal over-expression of endogenous NgR antagonist | From birth | Increased 5-HT axon sprouting caudal to the lesion site (28 days) | Improved BMS score and reduced footfall using grid runway test (28 days) | (Hirokawa et al., 2017) |
| Blocking extrinsic inhibitors such as chondroitin sulfate proteoglycans (CSPGs) | | | | | | |

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**Table 2** (*continued*)

| Strain, species (gender) | SCI model | Approach | Time (pre or post-SCI) | Anatomical finding (time post-SCI) | Functional recovery (time post-SCI) | Reference |
|---|---|---|---|---|---|---|
| Adult wistar rats (males) | C4 dorsal column crush | Repeated intrathecal ChABC infusion | Immediately post- injury | Increased 5-HT axon sprouting caudal to the lesion site (28 days) | No changes in pain response (28 days) | (Barritt et al., 2006) |
| Adult sprague-dawley rats (males) | T10 dorsal over-hemisection | Intrathecal thermostabilized chABC and NT-3 administration | Immediately post- injury | Increased 5-HT axon sprouting caudal to the lesion site (28 days) | Improved locomotion using Catwalk including stride length (42 days) | (Lee et al., 2010a) |
| Adult lister hooded rats (males) | C4 dorsal hemisection | Repeated intrathecal ChABC infusion and rehabilitation | 4 weeks post-injury | Increased 5-HT axon sprouting rostral to the lesion site (28 days) | Improved recovery of skilled paw reaching, ladder and beam walking (28 days) | (Wang et al., 2011a) |
| Adult sprague-dawley rats (females) | C2 lateral hemisection | Repeated intrathecal ChABC infusion and peripheral nerve autograft | Immediately post- injury | Increased 5-HT axon sprouting caudal to the lesion site (84 days) | Recovery of diaphragmatic muscle activity | (Alilain et al., 2011) |
| Adult sprague-dawley rats (females) | T8 severe contusion (250, kdyn) | CSPG inhibition via subcutaneous daily Intracellular Sigma Peptide (ISP) injection | 1 day post-injury | Increased 5-HT axon sprouting caudal to the lesion site (84 days) | Improved BBB score and reduced footfall using grid runway (49–77 days) and urinary function (84 days) | (Lang et al., 2015) |
| Adult sprague-dawley rats (females) | C2 lateral hemisection | Repeated intrathecal ChABC infusion | 1.5 years post-injury | Increased 5-HT axon sprouting caudal to the lesion site (1.5 years) | Improved breathing (1.5 years) | (Warren et al., 2018) |
| Promoting a Pro-regenerative environment by transplantation of neural precursors (NPs) and mesenchymal cells (MCs) | | | | | | |
| Adult C57BL/6 J mice (males) | T13 lateral hemisection | Transplantation of NPs or MCs caudal to the lesion | 14 days post-injury | Increased 5-HT axon sprouting caudal to the lesion site (42 days) | Improved BMS score and other motor performance (posture, foot-fault, hindlimb flexion and grip, 42 days) | (Boido et al., 2009) |
| Adult CBH-rnu/Arc, athymic nude, rats (females) | T9/T10 moderate/severe contusion (200, kdyn) | Implantation of hMPC bilateral to the lesion site | 7 days (sub-acute) or 30 days (chronic) post-injury | Increased 5-HT axon sprouting within the lesion site (60 days) | Acute implantation: Improved BBB score including weight support and coordination (35–77 days) Chronic implantation: Improved BBB score including weight support and coordination (56–77 days) | (Hodgetts et al., 2013) |
| Adult sprague-dawley rats (females) | T9 severe compression | Transplantation of naive or Neurogenin 2- expressing hENPs rostral, caudal and within the lesion site | 7 days post-injury | Increased 5-HT axon sprouting caudal to the lesion site (28 days) | Restored weight support using open field, improved fine motor control and reduced footfall using grid runway test (28 days) | (Perrin et al., 2010) |
| Adult fischer rats (females) | T4 transection | Implantation of BS-NSCs | 14 days post-injury | Increased graft-derived 5-HT axon regeneration caudal to the lesion site (77 days) | Recovery of basal cardiovascular parameters, and alleviation of autonomic dysreflexia (77 days) | (Hou et al., 2013) |
| Promoting a Pro-regenerative environment by transplantation of schwann cells (SCs) | | | | | | |
| Adult Fischer, rats (females) | T8 moderate contusion (10 g rod dropped from 25 mm height) | Implantation of PST-overexpressing SCs within the lesion site | 7 days post-injury | Increased 5-HT axon sprouting caudal to the lesion site (77 days) | Improved BBB score (21–63 days) and foot placement using grid runaway test (28–56 days) | (Ghosh et al., 2012) |
| Adult C57BL/6 J mice (females) | T8 complete compression | Implantation of PSA-NCAM-expressing SCs 0.5 mm rostral to the lesion site | Immediately post- injury | Increased 5-HT axon regeneration caudal to the lesion site (28 days) | Improved BMS score (21 and 28 days) | (Papastefanaki et al., 2007) |
| Adult Fischer rats (females) | T8 moderate contusion (10 g rod dropped from 12.5 mm height) | Implantation of neurotrophin and ChABC-expressing SCs within the lesion site | 7 days post-injury | Increased 5-HT axon sprouting caudal to the lesion site (91 days) | Improved BBB score (91 days), improved coordination using CatWalk gait analysis (63 days), reduced mechanical and thermal allodynia using von Frey and Hargreaves method (35–91 days) | (Kanno et al., 2014) |
| Adult Fischer rats (females) | T8 moderate contusion | Implantation of SCs combined with increase in cAMP within the lesion site | 7 days post-injury | Increased 5-HT axon sprouting caudal to the lesion site (77 days) | Improved BBB score and reduced footfall using grid runway (28–63 days) | (Pearse et al., 2004) |
| Increasing the Pro-regenerative capacity of injured neurons | | | | | | |

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**Table 2** (*continued*)

| Strain, species (gender) | SCI model | Approach | Time (pre or post-SCI) | Anatomical finding (time post-SCI) | Functional recovery (time post-SCI) | Reference |
|---|---|---|---|---|---|---|
| Adult C57BL/6 J mice (females) | T7 dorsal over-hemisection | Subcutaneous administration of PTEN antagonist peptide | 2–14 days post-injury | Increased 5-HT axon sprouting caudal to the lesion site (35 days) | Improved BMS score and reduced footfall using grid runway (14–35 days) | (Ohtake et al., 2014) |

Key: Kilodynes (kdyn), Basso Mouse Scale (BMS), short hairpin RNA (shRNA), Glial fibrillary acidic protein (GFAP), Vimentin (VIM), Basso, Beattie and Bresnahan (BBB) locomotor scale, Polysialyltransferase (PST), human mesenchymal precursor cell (hMPC), embryonic brainstem-derive neural stem cell (BS-NSC), Chondroitinase (ChABC), human embryonic neural progenitors (hENPs), neural precursors (NP), Nogo-66 receptor (NgR), Phosphatase and tensin homolog (PTEN), Not stated (NS), not assessed (NA), Chondroitin sulfate proteoglycans (CSPG), Intracellular sigma peptide (ISP), Neurotrophin-3 (NT-3).

clinically applicable for chronically injured SCI patients.

Some studies have combined cellular transplantation with strategies to concomitantly stimulate intrinsic pro-regenerative pathways, via elevation of cyclic adenosine monophosphate (Pearse et al., 2004), neurotrophin and chondroitinase (Kanno et al., 2014), to achieve a synergistic effect on 5-HT axon sprouting. However, whether targeting of intrinsic pro-regenerative pathways alone can stimulate 5-HT axon regeneration after SCI has thus far only been addressed in a single study. In this report, pharmacological targeting of mammalian target of rapamycin (mTOR) signaling led to increased 5-HT axon sprouting caudal to the lesion, associated with improved functional recovery after T7 dorsal hemisection of the mouse spinal cord (Ohtake et al., 2014).

The reader is directed to Table 2, which presents examples of pre-clinical treatments that have led to sprouting and regeneration of 5-HT axons caudal to the lesion site, associated with functional recovery after SCI. While improvement in functional recovery is essential for translational research, future studies should precisely uncover the source of new 5-HT axon outgrowth caudal to the lesion site to avoid misinterpretation of these findings. To this end, *in vivo* two-photon imaging of 5-HT axons is a reliable approach to discriminate between sprouting and regenerating axons. In addition, most therapeutic strategies report an association between increased 5-HT axon re-growth and functional recovery, without specifically examining 5-HT neurotransmission. It is of central importance to uncover which pro-regenerative pathways are activated in 5-HT neurons upon application of any therapeutic strategy. It is also currently unclear whether regenerated axons can maintain normal 5-HT neurotransmission within the injured spinal cord. The latter is particularly important given that 5-HT directly promotes non-serotonergic axon regeneration after injury in invertebrates, as further discussed below.

## 6. Direct role of 5-HT in promoting axon regeneration in invertebrates

The presence of 5-HT in single-celled eukaryotes, including *Tetrahymena,* suggests that it is one of the earliest evolutionary signaling molecule [for review see (Csaba, 2015)]. Indeed, both 5-HT and TrP promote cell growth in *Tetrahymena* (Csaba and Németh, 1980; Csaba et al., 1979), whilst 5-HT also enhances ciliary regeneration in deciliated *Tetrahymena* (Darvas et al., 1988). In *C. elegans*, 5-HT directly modulates axon regeneration following injury (Alam et al., 2016). Transcriptomic analysis identified tryptophan hydroxylase 1 (*tph-1*), which is responsible for 5-HT synthesis in the periphery, among several other genes that positively regulate axon regeneration in somatosensory neurons (Chen et al., 2011). A loss-of-function mutation in the *tph-1* gene results in impaired axonal regeneration in multiple neuronal types in *C. elegans* and this impaired regeneration is rescued upon exogeneous 5-HT treatment (Alam et al., 2016). In fact, axon regeneration in *C. elegans* depends on ectopic 5-HT synthesis in axotomized non-serotonergic neurons (Alam et al., 2016). Similarly, in adult zebrafish, exogeneous 5-HT treatment promotes motoneuron regeneration after complete section of the spinal cord (Barreiro-Iglesias et al., 2015). Intraperitoneal 5-HT injection increases the number of newly generated motoneurons caudal to the lesion site, a region that is typically devoid of descending 5-HT axons (Barreiro-Iglesias et al., 2015). Interestingly, exogeneous 5-HT treatment does not promote motoneuron growth in intact animals, suggesting that injury-induced signaling is necessary for the pro-regenerative effect of 5-HT in zebrafish (Barreiro-Iglesias et al., 2015). In contrast, a recent study in lampreys (a vertebrate) found that 5-HT inhibits axonal re-growth in identifiable descending neuron after spinal cord transection without affecting locomotor recovery (Sobrido-Cameán et al., 2019). Reports of invertebrates (*Helisoma* snail and *Lymnaea stagnalis*) and lower vertebrates (goldfish) also suggest an anti-regenerative role of 5-HT in neurite extension, which may be due to the use of different model systems, activation of disparate 5-HT receptors and diverse target neurons [for reviews see, (Sobrido-Cameán et al.,

2018; Trakhtenberg and Goldberg, 2012)].

*In vitro* studies using various cell lines (Fricker et al., 2005; Homma et al., 2006; Severin and Kondratyev, 1988), primary fetal mouse thalamic neurons (Persico et al., 2006), and embryonic rat hippocampal neurons (Rojas et al., 2014) support a pro-regenerative role of 5-HT on neurite outgrowth, although these reports do not exclude the possibility that the observed effects may be due to a role of 5-HT in neuronal differentiation. *In vivo*, inhibiting 5-HT synthesis between E12 – E17 in rats using PCPA, a tryptophan hydroxylase inhibitor, reduces dendritic arborizations and pyramidal neuron complexity within the somatosensory cortex (Vitalis et al., 2007). Transgenic mice with depleted central 5-HT ($TpH2^{-/-}$, $VMAT2^{sert\text{-}cre}$ and $VMAT2^{pet1\text{-}cre}$) display mild to severe post-natal growth retardations, depending on the extent of 5-HT reduction in the brain (Narboux-Nême et al., 2013). Also, in transgenic mice, elevation of central 5-HT ($MAOA^{-/-}$) results in altered neurite patterning during CNS development without gross axon abnormalities (Cases et al., 1995; Cases et al., 1996; Upton et al., 1999).

Overall, *in vitro* and *in vivo* experimental findings from both invertebrates and vertebrates, including mammals, highlight 5-HT-mediated stimulation of non-serotonergic neurite regeneration following injury. Interestingly, another line of evidence that also lends support to 5-HT-mediated enhancement of neurite outgrowth is during adult neurogenesis. For example, reduction of hippocampal 5-HT neurotransmission, by 5-HT neurotoxin (5,7-dihydroxytryptamine) injection in the dorsal and medial raphe nuclei, decreases neurogenesis in the adult rat hippocampus (Brezun and Daszuta, 2000). Furthermore, inhibition of 5-HT synthesis by PCPA treatment reduces dendritic length, prevents dendritic spine formation, and triggers synaptic loss (Faber and Haring, 1999; Mazer et al., 1997; Okado et al., 1993; Yan et al., 1997), associated with diminished hippocampal neurogenesis in adult rats (Huang and Herbert, 2005; Jha et al., 2006). On the other hand, elevation of 5-HT neurotransmission through chronic treatment with antidepressants increases hippocampal neurogenesis in adult mice (Encinas et al., 2006; Klempin et al., 2010; Santarelli et al., 2003) and rats (Malberg et al., 2000). Altogether, these findings suggest that the pro-regenerative role of 5-HT in promoting neurite outgrowth may be evolutionarily conserved. Increased 5-HT neurotransmission may not only address the neurochemical imbalance after SCI but may also stimulate synaptic re-modelling in the lesioned spinal cord. Uncovering molecular mechanisms responsible for 5-HT-mediated stimulation of axonal re-growth may be instrumental in the development of novel pro-regenerative therapies to promote CNS axon regeneration.

## 7. Future directions

There are several features of the 5-HT system that warrant further investigations regarding their mechanistic roles in SCI pathophysiology.

***Firstly***, 5-HT axons not only display good resistance within an injured milieu but also actively sprout and regenerate after CNS insult. Though increased expression of growth-promoting receptors, reduced expression of growth-inhibiting receptors, and lack of synaptic contacts by 5-HT axons may be potential mechanisms that explain these attributes, actual evidence backing these possibilities is currently lacking. Cell-specific transcriptomic and proteomic studies of 5-HT neurons are powerful approaches that may unveil the explicit molecular mechanisms responsible for 5-HT axonal sprouting and regeneration following CNS injury. Transgenic mice expressing enhanced green fluorescent protein under serotonin transporter (*Slc6a4*-EGFP) may indeed be used, in combination with flow cytometry to isolate 5-HT neurons and carry out cell-specific transcriptional analysis. Alternatively, and to avoid alterations in gene expression due to enzymatic digestion, excess processing, and severe tissue disruption, one can also use the Slc6a4 bacTRAP transgenic mice and utilize translating ribosome affinity purification method for direct purification and high throughput analysis of translating mRNAs from 5-HT neurons (Dougherty et al., 2013). These studies may be done at the level of both axon and soma, as shown recently in retinal ganglion cells (Shigeoka et al., 2016).

***Secondly***, 5-HT axons are sensitive to altered 5-HT neurotransmission in the intact CNS. Whether chronic elevation of 5-HT neurotransmission after SCI would induce additional axonal sprouting and regeneration associated with functional improvements needs to be investigated further. To this end, the non-invasive nature of altered dietary TrP intake is an attractive non-pharmacological approach to effectively modify central 5-HT neurotransmission after SCI. Alternatively, chronic treatment with antidepressant drugs that has been known for over 3 decades to elevate 5-HT neurotransmission [for review see (Willner, 1985)] can also be used to increase 5-HT neurotransmission following SCI. Of equal importance, while elevated 5-HT neuronal activity may contribute to a neurotransmitter-mediated increase in 5-HT axonal sprouting, other specific molecular players driving this process need to be uncovered. This could also be achieved using cell-specific transcriptomic analysis in *Slc6a4*-EGFP or Slc6a4 bacTRAP mice upon chronic elevation of 5-HT neurotransmission after SCI.

***Thirdly***, 5-HT may have a non-autonomous pro-regenerative effect. Whether increased 5-HT neurotransmission also causes sprouting and/or regeneration of non-serotonergic axons after SCI awaits further investigation. In support of this, chronic (9 weeks) treatment with fluoxetine in rats promote sprouting of corticospinal tract axons after C4 dorsal funiculus crush (Scali et al., 2013). This can be investigated by injecting anterograde tracers into motor cortex and dorsal root ganglion to trace corticospinal tract and sensory axons, respectively (Hilton et al., 2019). Adeno-associated virus (AAV) expressing GFP or red fluorescent protein fluorophores could also represent an alternative to other classical tracers such as biotinylated dextran amine.

***Fourthly***, it is likely that not all 5-HT neurons have the same capacity to re-growth following SCI. This suggests that intrinsic factors present in some 5-HT neurons, but not in others, might limit their regenerative ability. One mean to answer this question, is to intrathecally inject retrograde tracers caudal to the lesion site in *Slc6a4*-EGFP mice after experimental manipulation that causes 5-HT axon regeneration. Thus, 5-HT neurons with regenerating axons (EGFP/retrograde tracer dual positive) could be isolated and their transcriptomic profile compared with non-regenerating (EGFP only) 5-HT neurons. It is also possible that increased regenerative ability of 5-HT neurons is target driven. A recent study of the mouse dorsal raphe nucleus (B6 – B7) suggests that some serotonergic axons projecting to the ventral tegmental area co-release glutamate that in turn triggers dopamine release in the nucleus accumbens and promotes conditioned place preference (Wang et al., 2019). Whether particular subtype of 5-HT neurons within other raphe nuclei also co-release other neurotransmitter that may influence their regenerative response await further study.

***Finally***, despite their unusual ability to spontaneously sprout after SCI, 5-HT axons fail to cross the lesion site after transection of the spinal cord. Studies in the last decade have shown a notable increase in axonal regeneration following manipulation of multiple intrinsic signaling pathways, including those involving mTORC1 (Park et al., 2008), signal transducer and activator of transcription 3 (Moore et al., 2009) and extracellular signal-regulated kinase (O'Donovan et al., 2014). In fact, corticospinal axons with elevated mTORC1 pathway activity can cross the lesion site after SCI (Liu et al., 2010). Given the natural sprouting response of 5-HT axons rostral to the lesion, one could hypothesize that activation of these intrinsic pro-regenerative pathways in 5-HT neurons may be an effective approach to enhance their long-distance axon regeneration across the lesion site after complete SCI. Previous findings suggest that genetic attenuation of extracellular myelin inhibitors and chemo-repulsive axon guidance molecule (class 3 Semaphorins) do not promote regeneration of 5-HT axons after complete section of the mouse spinal cord (Lee et al., 2010b), suggesting that boosting the intrinsic machinery may be more beneficial to enhance 5-HT axonal regrowth following SCI. To selectively activate these regenerative pathways in 5-HT neurons, AAV encoding Cre-dependent manipulations of

these pathways can be injected directly in the raphe nuclei of SERT-Cre (B6.129(Cg)-Slc6a4tm1(cre)Xz/J) mice. Remarkably, other strategies that promote plasticity of the 5-HT system by modulating extrinsic factors, such as physical rehabilitation, may also provide an attractive strategy to further improve functional recovery following SCI. Targeting the 5-HT system thus holds great potential to influence the pathophysiological response after SCI.

## Conflict of interest statement

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

## Authors' contributions

F.E.P. and H.N.N. contributed to the writing of the manuscript.

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