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Serotonergic influences on locomotor circuits

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Serotonin (5-HT) neurons can be activated from the Mesencephalic Locomotor Region (MLR) to produce release of 5-HT in the spinal cord, and MLR evoked locomotion can be blocked by a 5-HT_7 receptor antagonist. 5-HT control shifts during development from control of coordinating interneurons in neonates to influences on afferent input in adults. Pharmacotherapy involving 5-HT receptor agonists has progressed to clinical trials on human subjects with spinal cord injury (SCI). Work on plasticity of the 5-HT system shows that SCI results in changes in 5-HT receptor protein and mRNA, and that these changes can be reversed by exercise or by replacement of 5-HT with intraspinal grafts. Recent data on cross-talk and heterodimerization of these receptors suggest new mechanisms for plasticity.

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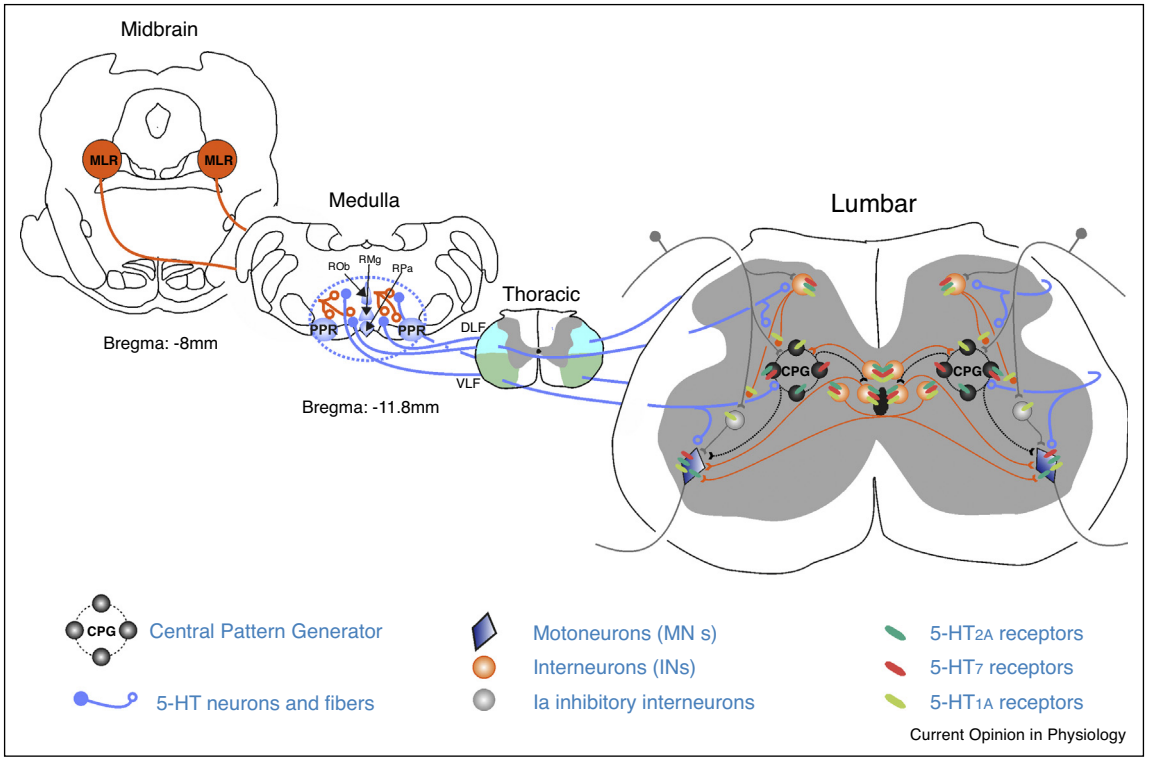
Basic mechanisms of serotoninergic motor control in mammals

The importance of serotonin in the control of locomotion has been emphasized in recent reviews [1,2,3•,4,5,6•]. Serotonergic pathways are included in the reticulospinal (RS) pathways activated by the locomotor signals from higher structures of the brain. The RS neurons are activated by the Mesencephalic Locomotor Region (MLR), which includes the cuneiform nucleus (CnF), the pedunculopontine nucleus (PPN), and surrounding structures, and which receives input and control from forebrain as well as diencephalic, limbic and basal ganglia structures which engage the midbrain locomotor areas for the full range of locomotor tasks. We have summarized the 5-HT system involved in locomotor control in Figure 1. Recent evidence shows that the 5-HT RS neurons are activated when locomotion is initiated by MLR stimulation. Noga and Opris [7] demonstrated in cats that monoaminergic neurons located in the nuclei of origin of descending serotonergic pathways innervating the spinal cord are activated during MLR-evoked locomotion. Locomotion induced by MLR stimulation resulted in the expression of the activity-dependent marker Fos in 5-HT neurons of nucleus raphe magnus (RMg) and the Parapyramidal Region (PPR) of the medulla [7]. In rats a component of the MLR (CnF) projects to descending 5-HT neurons in the medulla [8–11], whereas evidence for such a projection from PPN is less clear. CnF has recently been shown to be the main component of the MLR for inducing locomotion in mice [12••]. Josset *et al.* [12••] found that photostimulation of glutamatergic CnF neurons initiate and accelerate locomotion, whereas glutamatergic and cholinergic PPN contribute to slow-walking gaits and locomotor arrests. Other recent studies using genetic tools in mice have also implicated the CnF, but show evidence for a greater role for PPN neurons [13,14]. These studies confirm work in the cat model, which implicate the CnF as the effective site for producing locomotion [15•]. Stimulation of the MLR results in release of 5-HT in the cat spinal cord [16•]. This is direct evidence that MLR stimulation activates descending 5-HT neurons. Other recent evidence supporting this conclusion is provided by the fact that voluntary locomotion in intact adult rats and fictive locomotion induced by MLR stimulation in adult decerebrate rats is blocked by an intrathecal 5-HT_7 receptor antagonist [17]. In contrast to neonatal animals, in adult rats there is no effect on coordinating interneurons by this receptor, but the effect is mediated by this receptor shifts during development [18] to influences on afferent input. These studies support previous findings that locomotion is associated with 5-HT release in the spinal cord of rats [19,20], 5-HT and its agonists can produce locomotion [21–23], and 5-HT neurons grafted into the spinal cord facilitate locomotion [24–28]. We suggest that MLR activation of descending 5-HT neurons of the medulla normally occurs when locomotion is initiated, resulting in CPG activation involving 5-HT_7, 5-HT_{2A}, and 5-HT_{1A} receptors (Figure 1).

The role of 5-HT receptors in locomotor control

The important role played by 5-HT in the initiation of locomotion has been advanced by extensive research efforts using isolated neonatal rodent preparations (reviewed in Refs. [23,29–32]). The descending 5-HT neurons in B1–B3 appear to be recruited during activation of the brainstem locomotor region (Figure 1), and

Figure 1



Schematic view of neuronal projections from the MLR toward the serotonergic raphe nuclei (ROb—raphe obscurus; RPa—raphe pallidus; RMg—raphe magnus) and the PPR (Parapyramidal Region). Axons of serotoninergic (5-HT) neurons descend via the ventrolateral funiculus (VLF) to innervate lumbar spinal locomotor interneurons comprising the central pattern generator (CPG) and lumbar motoneurons (MNs) and via DLF to innervate dorsal horn interneurons. Schematic drawing of brain and spinal cross-sections based on Paxinos 1995 'The rat Nervous system' 2nd edition [83]. Receptor distribution is based on observations in the Jordan lab as well as the Sławińska lab [17,23,84].

stimulation of 5-HT neurons in the medulla elicits locomotion in isolated rat brainstem and spinal cord preparations [18] that is blocked by spinal application of 5-HT antagonists. Husch *et al.* have developed a means of studying fictive locomotion *in vitro* using perforated patch [33], and they went on to show intrinsic properties and serotonergic modulation of commissural interneurons (CINs) change during postnatal development in mice so that by P14–16, when mature walking emerges, serotonin can induce plateau potential capability [34]. Genetically identified V2a locomotor neurons display intrinsic membrane potential bistability in adult mice [35] but not in younger animals. Thus, the properties of neurons examined in neonatal rodent preparations may not be typical of what happens in adult animals (see Refs. [17,36]).

Constitutively active 5-HT_{2C} receptors (active in the absence of 5-HT) in rat motoneurons, which emerge after SCI, have been implicated in the recovery of locomotion after injury [37,38]). A neutral antagonist of 5-HT_{2C} receptors was unable to block this effect, but an inverse agonist was effective. This is a feature of constitutive activity, which appears due to plasticity after chronic SCI. This is also suggested to account for the emergence of spasticity after SCI. It has also been suggested that the appearance of increased numbers of interneurons expressing AADC (Aromatic L-Amino Acid Decarboxylase Cells), which can produce 5-HT when exogenous 5-HT precursors are available [39–41], normalizes 5-HT function after SCI.

A role for 5-HT_{1A} receptors in the control of locomotion is supported by numerous studies on rats using 8-OH-DPAT, which has affinity for both 5-HT_{1A} and 5-HT_7 receptors [21–23,42,43]. A clear demonstration of the contribution of 5-HT_{1A} receptors to locomotor-like activity was carried out by Landry *et al.* [44], who showed 8-OH-DPAT produced locomotor-like activity in the spinal cord of 5-HT_7 receptor knockout mice. Some cat spinal interneurons that are active during locomotion possess 5-HT_{1A} and 5-HT_7 receptors [45]. The interest in the ability of these 5-HT receptors to induce locomotion after spinal cord injury has led to recent attempts to use the 5-HT receptor agonist Buspirone in human subjects with spinal cord injury [46–48]. Buspirone has been shown to facilitate locomotor recovery in spinal mice [49•] in combination with training. The precise mode of action of Buspirone in these cases is uncertain, because it is an agonist of 5-HT_{1A}, 5-HT_2 and 5-HT_7 receptors

[46,49•,50]. Spinalon™ is a therapy based on the 5-HT agonist Buspirone with additional components (carbidopa/levodopa) that has been tested recently for efficacy and safety [6•,50,51•]. However, the results of Spinalon™ treatment are ambiguous because 66% patients did not respond to treatment even if in the other completely paralyzed individuals promising improvement of rhythmic leg activity was observed [51•]. Efforts to enhance the efficacy of pharmacological therapy using other modalities of treatment, including locomotor training and epidural stimulation, have been recently reviewed [6•,50].

Serotonergic plasticity related to locomotor recovery after SCI

Sprouting of 5-HT fibers

It is uncertain what contribution 5-HT makes to the control of locomotion in humans, but extensive recent research in rodents has provided an appreciation of the importance of 5-HT in these species. Of particular interest are recent studies that show a strong correlation between recovery of locomotor function and the presence of 5-HT fibers and terminals in the lumbar cord [52–55]. Included in some of these studies is the fact that locomotor training or exercise promotes pro-locomotor plasticity in the 5-HT system.

Upregulation of 5-HT receptors

A consideration of the contribution of 5-HT receptors to the recovery of locomotor function after injury requires attention to the injury-induced plasticity. Considerable interest has been focused on upregulation of specific 5-HT receptors in rats, including, 5-HT_{1A}, 5-HT_{2A}, 5-HT_{2C} or alternative splicing of 5-HT_{2C} [37,43,56•]. All of these receptors are implicated in the control of locomotion (reviewed in Refs. [2,57]).

Activation of spinal 5-HT_{1A} receptors is thought to facilitate locomotion in rodents [3•,44], and upregulation of 5-HT_{1A} receptors after spinal cord injury is a prominent feature of the response to spinal cord injury [43,56•]. The upregulation in 5-HT_{1A} receptors following spinal cord transection does not occur when the cord in also 'isolated' (deafferented), suggesting a role for afferents from the limb in the expression of these receptors [43]. The significant role of afferents from the limb was demonstrated in upright walking in spinal rats [58]. Considering that 5-$HT_{1A/7}$ agonists (8-OH-DPAT) improve locomotion in the horizontal posture but impair locomotion in the upright posture, it can be suggested that a proper balance of afferent feedback from the foot and descending supraspinal inputs through 5-HT_{1A}/5-HT_7 receptor activation is necessary for optimal locomotor recovery [58].

Upregulation of 5-HT receptors after SCI in rats has been confirmed recently by Ganzer *et al.* [56•], who demonstrated that 5-HT pharmacotherapy (quipazine and 8-OH-DPAT) and bike exercise normalized 5-HT receptors (5-HT_{2A}, 5-HT_{1A}) and dendritic atrophy after SCI. Further research has focused on SCI and exercise [59] or restoration of 5-HT innervation [60] effects on receptor mRNA in motoneurons of rat hindlimb muscles. It was demonstrated that graft origin 5-HT innervation in the spinal cord of paraplegic rats could reverse changes in gene expression of 5-HT_{2A} and 5-HT_7 receptors induced by SCI in motoneurons innervating hindlimb muscles but not those innervating tail muscles [60].

5-HT_{2A} receptors control of K^+–Cl^- cotransporter (KCC2)

5-HT_{2A} receptors are implicated in the control of KCC2, the main Cl^- extruder in mature rat neurons [61•]. It is known that KCC2 is responsible for maintaining the intracellular chloride concentration. Downregulation of KCC2 induced by spinal cord injury in rats, particularly in motoneuron membranes, causes a reduction of the strength of postsynaptic inhibition by depolarizing the Cl^- equilibrium potential [62]. Vinay's group demonstrated that activation of 5-HT_{2A} receptors in paraplegic rats, which hyperpolarizes the reversal potential of inhibitory postsynaptic potentials (IPSPs), increases the cell membrane expression of KCC2 and restores endogenous inhibition [61•]. Tashiro *et al.* demonstrated that treadmill training that upregulated endogenous brain-derived neurotrophic factor (BDNF) expression also induced post-translational modification of KCC2 in the rat lumbar enlargement [63]. Thus, neural plasticity expressed in the upregulation of KCC2 induced by activation of 5-HT_{2A} receptors or by treadmill training, has therapeutic potential in the treatment of altered chloride homeostasis and may provide a new strategy for functional rehabilitation of locomotor movements.

Spasticity—an effect of hyperexcitability as a result of post-injury plasticity

Motoneuron excitability after SCI is regulated not only by restoring endogenous inhibition via the 5-HT_{2A} receptor but by upregulation of the constitutively active unedited form of the 5-HT_{2C} receptor [37]. Tysseling *et al.* [64] showed that constitutively active 5-HT_{2C} contributes to reflex hyperexcitability of sacral segments of spinal cord in mice with incomplete chronic thoracic spinal cord injury in *in vitro* conditions. However, their *in vivo* investigations in these same mice did not include tests of constitutive activity of 5-HT_{2C} receptors on reflexes induced by electrical stimulation of the plantar surface of the hind paw. These hind limb reflexes were not significantly modulated by SSRI or baclofen treatment, showing the increased amplitude after partial SCI was not altered by increased the release of 5-HT or by increased inhibition. This would be consistent with constitutive activity of 5-HT receptors on hind limb motoneurons, but further experiments will be necessary to determine whether any excitability changes detected in limb motoneurons is due to plasticity in 5-HT_{2C} receptors, because the effects of a

neutral antagonist and an inverse agonist of 5-HT_{2C} receptors were attempted only on reflexes in isolated sacral spinal cords from the same animals. Recent data [65] revealed that excitatory spinal interneurons are recruited by sensory input to generate persistent neural activity after sacral SCI. In our experiments, tail and limb motoneurons respond differently to chronic SCI with regard to changes in 5-HT gene expression [60]. These experiments raise new questions about the detailed forms of plasticity induced by chronic SCI, and point out that constitutive activity of 5-HT receptors in motoneurons (see Ref. [66]) is not the sole basis for altered 5-HT action.

Crosstalk as a mechanism of neural plasticity

Interest in intermittent hypoxia (IH) as a means of enhancing functional recovery after spinal cord injury has been prompted by findings on the control of phrenic motoneurons after spinal cord injury. IH triggers episodic serotonin release near motoneurons, activating serotonin receptors and triggering a cellular cascade leading to increased BDNF synthesis, TrkB activation, and downstream events that enhance synaptic inputs to relevant motoneurons. Crosstalk between $5HT_{2A}$ and 5-HT_7 receptors occurs such that 5-HT_7 receptor activation can suppress the 5-HT_{2A} mediated response to intermittent hypoxia in rats [67–69]. Recent clinical trials in patients have revealed that IH can be effective in enhancing locomotor recovery after SCI [70•]. Further work is needed to determine the role, if any, of serotonin in the effect of IH on walking.

Potential role of 5-HT receptor heterodimerization

Recently, a considerable body of evidence indicates that cross-talk interactions between various 5-HT receptors can contribute to the regulation of various physiological functions, normally and in pathology [71,72]. One pair of such receptors are 5-HT_{1A} and 5-HT_7, which can form heterodimers *in vitro* and *in vivo* [73]. This results in the weakening of Gi-protein activation mediated by 5-HT_{1A} receptors, whereas the functions of 5-HT_7 are not significantly affected. There are data demonstrating a critical role of these receptors in control of locomotion in mammals, so it is possible that the heterodimer structure is involved in locomotor function as well. Our recent investigations demonstrated that using 8-OH-DPAT (agonist of 5-$HT_{1A/7}$ receptors) is more effective in enhancing recovery of locomotor function in paraplegic rats than LP211 (agonist of 5-HT_7 receptors; gift from Marcello Leopoldo). Activation of both receptors together with 8-OH-DPAT has a more potent effect on locomotion induction than activation of 5-HT_7 receptors alone (preliminary observation), suggesting that some interaction between these receptors may occur. If they form heterodimers then the activation of 5-HT_7 receptors may alter the efficacy of 5-HT_{1A} receptors as was shown in other pathological conditions [72,74,75].

It is known that 5-HT_{1A} receptors play an important role in the regulation of neuronal development and plasticity [72]. 5-HT_7 receptors are involved in the posttranscriptional regulation of the functional state of 5-HT_{1A} receptors [71]. It is also known that 5-HT_{1A} receptors seem to be regulated by the metabotropic 5-HT_{2A} receptors [76]. Interplay between 5-HT_{2A} and 5-HT_{1A} receptors was demonstrated by chronic [77,78] and acute [79,80] activation of 5-HT_{2A} receptors, which produced considerable reduction in 5-HT_{1A} receptor functional activity in rats. Other receptors important in locomotion that can form heteromers are the 5-HT_{2A} and 5-HT_{2C} receptors. For example, colocalization of both 5-HT_{2A} and 5-HT_{2C} is described in phrenic motoneurons of the cervical ventral horn. They are implicated in respiratory recovery following cervical hemisection [81], because 5-HT_{2A} receptors suppress the function of 5-HT_{2C} receptors. Thus, the interplay of 5-HT_7/5-HT_{1A}, 5-HT_{2A}/5-HT_{1A} and/or 5-HT_{2A}/5-HT_{1A} receptors might be involved in serotonin system plasticity. Such interactions need to be investigated in locomotion.

There are many other examples of interplay between different 5-HT receptors [82] that might be involved in neural plasticity associated with recovery of motor function induced by various rehabilitation strategies. However, this topic remains still controversial and needs to be investigated in more detail.

Conclusions

Recent evidence supports the hypothesis that 5-HT influences at the spinal level are required for control of locomotion. The 5-HT system is subject to plasticity after injury that can be normalized by activity or cell replacement therapy, and this may be associated with enhancement of locomotor recovery. Previously unknown properties of 5-HT receptors have been revealed, and they may contribute to the normal control of locomotion as well as to the response to spinal cord injury. Knowledge regarding the 5-HT receptors involved in locomotor control has led to clinical trials for restoration of function after injury. Further investigation is needed on all these fronts to maximize our ability to restore locomotion.

Conflict of interest statement

Nothing declared.

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