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Serotonergic modulation of spinal motor control

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Serotonin (5-HT) is a monoamine that powerfully modulates spinal motor control by acting on intrasynaptic and extrasynaptic receptors. Here we review the diversity of 5-HT actions on locomotor and motoneuronal activities. Two approaches have been used on *in vitro* spinal cord preparations: either applying 5-HT in the extracellular medium or inducing its synaptic release. They produced strikingly different results suggesting that the net effect of 5-HT depends on the identity of the activated receptors and their location. Recent findings suggest that moderate release of 5-HT facilitates locomotion and promotes the excitability of motoneurons, while stronger release inhibits rhythmic activity and motoneuron firing. This latter effect is responsible for central fatigue and secures rotation of motor units.

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Introduction

The role of serotonin (5-hydroxytryptamine, 5-HT) as regulator of motor patterns has been extensively studied during the past fifty years. Several publications have demonstrated that 5-HT has multiple effects on spinal motor circuits. In the present article, we review the literature demonstrating that exogenous application of 5-HT induces multiple and contradictory effects on spinal rhythmic activities and on motoneuron excitability. We argue that the heterogeneity of these actions only makes sense when considered under more physiological conditions: that is, when 5-HT is released from synapses and differentially activates receptors depending on their locations and affinities.

Organization of the serotonergic system

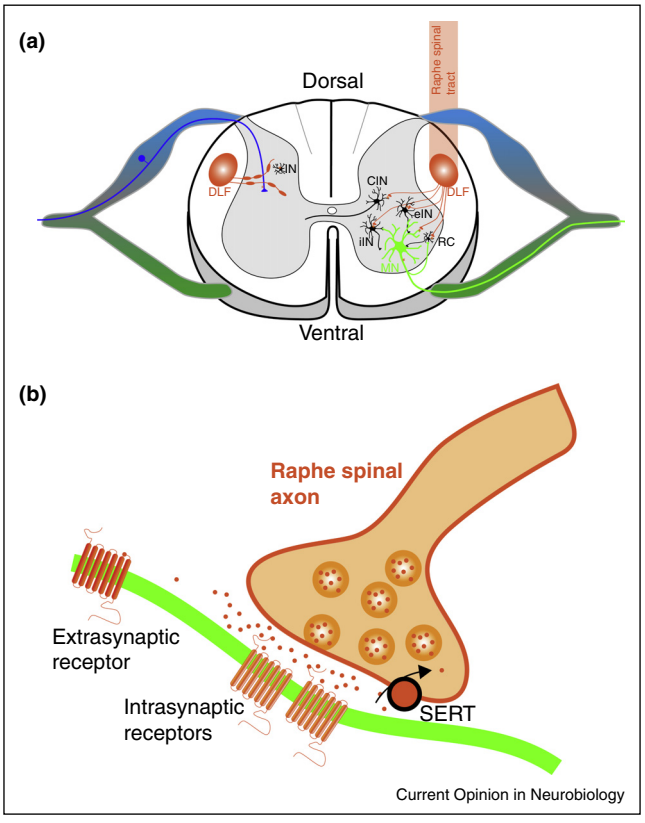
5-HT is a neurotransmitter that is mainly synthetized in neurons in the brainstem raphe nuclei. The raphe spinal pathways originate from *raphe obscursus*, *raphe pallidus*, *raphe magnus* and *raphe dorsalis* to terminate at all levels along the spinal cord [1,2]. In the dorsal horns, where sensory information is processed, serotonergic varicosities are characterized by non-synaptic contacts suggesting a paracrine mode of release [3]. By contrast, in the ventral horn, raphe spinal neurons make well-defined synaptic contacts on interneurons and motoneurons [4,5] (Figure 1). Once released, 5-HT binds to receptors in the 15-member serotonin receptor family. With the exception of 5-HT_3, 5-HT receptors are coupled to G proteins of the Gi/o, Gs and Gq subgroups [6]. This indicates that 5-HT can trigger a wide variety of regulatory actions. Indeed, in spinal cord neurons, 5-HT was shown to facilitate Na^+, Ca^{2+}, cations currents, to inhibit K^+, Ca^{2+}, Na^+ currents and to modulate synaptic transmission (see Perrier *et al.* [7•] for review). 5-HT receptors have different affinities for their natural ligand, the 5-HT_1 and 5-HT_7 receptors presenting the highest binding affinity (see Table 1 in Murray *et al.* [8] for an exhaustive review). Most serotonergic receptors are present in the spinal cord. They are expressed presynaptically and in the somato-dendritic compartments [7•,9–15]. Some of the receptors such as the 5-HT_{1A} and 5-HT_{2A} have been detected outside serotonergic synaptic innervation [16••,17], suggesting that they are activated by a background concentration of 5-HT present in the extracellular space and/or by spillover occurring during intense synaptic activity of the raphe spinal pathway. 5-HT is cleared from the extracellular space by a specific transporter (SERT) located in the terminals and varicosities of raphe spinal neurons (Figure 1b). The expression of SERT parallels the serotonergic innervation [18] suggesting that 5-HT is removed at different rates in distinct extracellular compartments of the spinal cord.

The heterogeneity of 5-HT receptors in terms of affinity, subcellular location and intracellular activated pathways makes it virtually impossible to predict how synaptic release of 5-HT may affect motor control. Interestingly, neurons in the raphe nuclei fire regularly at frequencies tightly correlated with motor activities [19]. This suggests that the amount of 5-HT released in the spinal cord varies in parallel with movement intensity. This important point indicates that 5-HT receptors are not activated together under physiological conditions. Instead the moderately active raphe spinal pathway may preferentially activate sub-synaptic and high affinity receptors while high levels of activity may also increasingly recruit low affinity and extrasynaptic receptors.

Serotonin differentially regulates rhythmic activity in the spinal cord

Locomotion, such as walking or swimming, is orchestrated by a subset of interneurons located in the ventro-medial

Figure 1



Organization of the raphe spinal pathway. **(a)** The raphe spinal tract located in the *dorsolateral funiculus* (DLF) releases 5-HT in a paracrine manner in the dorsal horn and makes well defined synaptic contacts on different neurons from the ventral horn. CIN: commissural interneuron; eIN: excitatory interneuron; iIN: inhibitory interneuron; RC: Renshaw cell; MN: motoneuron. **(b)** Once synaptically released 5-HT binds to intrasynaptic and extrasynaptic receptors before being recaptured by a specific transporter (SERT).

part of the spinal cord [20]. Most interneurons from this area, receive dense serotonergic synaptic innervation including the Renshaw cells that mediate recurrent inhibition of motoneurons [21,22] (Figure 1). These observations suggest that 5-HT modulates locomotor activity. In agreement, several studies demonstrated that bath application of 5-HT in *in vitro* spinal cord preparations triggers fictive locomotion characterized by rhythmic activities in muscles or in limb motor nerves [23,24]. However, the effects induced by 5-HT on locomotion are not straight forward and the heterogeneity of the observations reported reflects the complexity of the modulation. Fictive locomotion is usually characterized by parameters such as cycle period, amplitude of bursts of activity recorded in individual motor nerves, coordination between left and right or between ventral roots preferentially innervating flexor and extensor muscles.

Few studies reported that an exogenous application of 5-HT on the spinal cord increases locomotion speed. In zebrafish larvae, 5-HT decreases the interburst interval [25]. Similar findings were reported in the newborn rat where 5-HT significantly decreases the period of the locomotor cycle in a dose-dependent manner [23]. By contrast, most other studies performed in the lamprey, frog embryo, juvenile zebrafish, neonatal rat and mouse reported an inhibitory effect of 5-HT characterized by a dose-dependent increase of the cycle period [26–32]. There is a general agreement that this inhibitory effect is caused by the activation of 5-HT_1 receptors [26–28,33]. Exogenous application of 5-HT also increases the coordination between limbs and between flexor and extensor muscles [13,31,34–36]. These effects are mediated by 5-HT_2 and 5-HT_7 receptors [13,31,35]. The activation of serotonergic receptors in commissural interneurons increases their excitability by promoting L-type Ca^{2+} channels and by inhibiting N and P/Q type Ca^{2+} channels. This latter effect prevents the activation of Ca^{2+}-dependent K^+ channels, thus increasing the firing of commissural interneurons and thereby the left-right coordination [37,38]. At high speed, left-right coordination is secured by the recruitment of V2a interneurons [39]. 5-HT promotes the excitability of these excitatory interneurons that project to ipsilateral commissural interneurons [40–42]. Since the release of 5-HT increases with locomotion speed [19], we postulate that it contributes to the progressive recruitment of V2a interneurons thereby preventing a switch from trotting to galloping at high speeds.

The heterogeneity of the effects induced by exogenous application of 5-HT can be explained by the multiplicity of receptor subtypes that get activated simultaneously. In comparison, under physiological conditions, endogenous 5-HT differentially binds receptors with a preference for intrasynaptic and high-affinity subtypes. We believe synaptic release of 5-HT is necessary for understanding how 5-HT modulates locomotion. Only few studies have used such an approach. Recently, the group of Whelan evoked locomotion in a brainstem-spinal cord preparation by electrical stimulation of the brainstem and investigated the role of endogenous 5-HT by increasing its release with the Selective Serotonin Reuptake Inhibitor (SSRI) citalopram [27]. Compared to exogenous 5-HT, citalopram increases the concentration of the neuromodulator inside and near synapses. Not surprisingly, they found that the effects induced by the SSRI differed from those induced by exogenous application of 5-HT. Both approaches induce an increase in the duration of the locomotor cycle. However, contrary to exogenous 5-HT, citalopram decreases the coordination measured between left/right sides and between flexors and extensors. Pharmacological analysis suggests that the increase in cycle duration is mediated by 5-HT_1 receptors. By contrast, the activation of 5-HT_2 and 5-HT_7 receptors has an excitatory effect. Based on their findings, the authors

speculate that when released at low level in the synaptic cleft, 5-HT facilitates locomotion, while at higher level it exerts an inhibitory influence [27].

It would be interesting to determine the subcellular location of the different 5-HT receptors and in particular to establish the identity of intrasynaptic or extrasynaptic 5-HT receptors as their activation depends on the level and location of 5-HT release and degradation. It is possible that intrasynaptic and extrasynaptic receptors exert antagonist effects on locomotion. During low-level release of 5-HT, the intrasynaptic receptors would be preferentially activated, while the extrasynaptic receptors would be only activated during spillover. This hypothesis has not yet been tested for the neuronal networks that govern rhythmic activity. However, strong evidence suggests that such a mechanism controls the excitability of motoneurons [16••].

Serotonin differentially controls the excitability of motoneurons

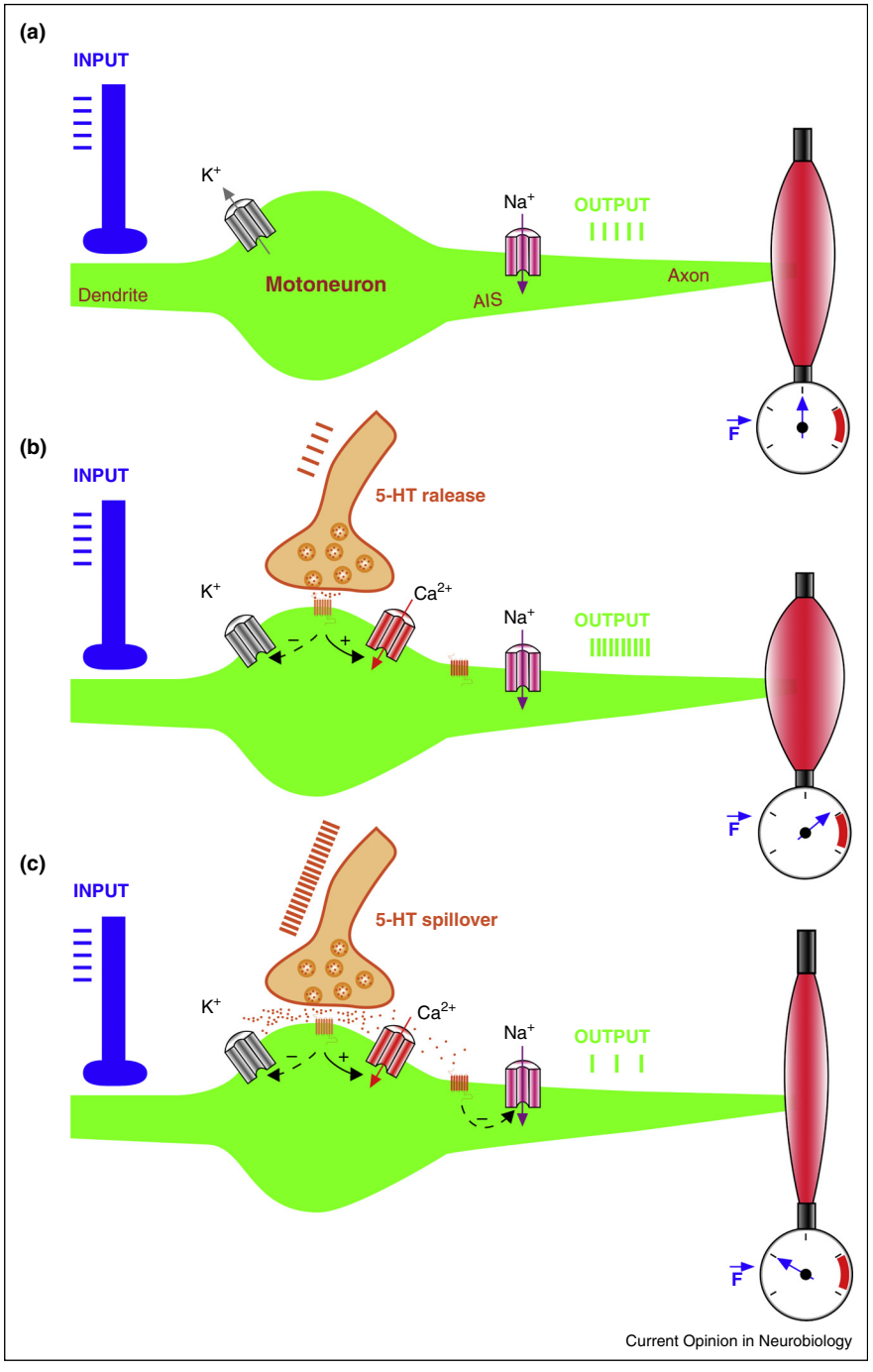
Recent experiments performed on humans showed that increasing the concentration of 5-HT in the central nervous system enhances the amplitude of monosynaptic spinal reflexes with the consequence that the force involved in precision tasks is increased [43•]. These observations suggest that 5-HT promotes the excitability of motoneurons. Experiments performed in animal slice preparations demonstrated that 5-HT depolarizes the membrane potential and increases the input resistance of motoneurons. These effects are caused by the activation of at least two distinct receptors. The activation of 5-HT_{1A} receptors inhibits leak K^+ channels [44,45] and the slow after hyperpolarization following action potentials mediated by Ca^{2+}-activated K^+ channels (SK) [46–49]. This latter action is both caused by the inhibition of the calcium current responsible for the activation of SK channels [46] and a direct inhibition of the SK channels [47]. The binding of 5-HT to 5-HT_2 receptors facilitates motoneurons by promoting persistent inward currents mediated by L-type Ca^{2+} channels [8,50] and Na^+ channels [51] and by facilitating Ih current [52].

Few publications that are often disregarded have shown that 5-HT also decreases the excitability of motoneurons. These effects are characterized by a hyperpolarization associated with an increase in membrane conductance [52,53].

Interestingly 5-HT has been also suspected to be responsible for the induction of central fatigue for many years. This form of motor fatigue characterized by a decreased ability to contract muscle fibers adequately during a motor activity is independent of muscle fatigue [54]. Several arguments based on experiments on animals and humans suggest a link of causality between 5-HT and fatigue. The 5-HT precursor tryptophan (Trp) crosses the blood brain barrier in competition with branched-chained amino-acids (BCAAs). Ingestion of a diet enriched in BCAAs decreases the amount of Trp available in the brain and thereby the rate of 5-HT synthesis. Interestingly, the diet improves physical performances of human subjects [55]. In agreement, systemic or intracranial injection of Trp accelerates the exhaustion of animals performing prolonged motor tasks [56,57] while blocking the conversion of Trp to 5-HT prevents the effect of Trp on fatigue [58]. In agreement, the oral intake of the 5-HT_{1A} receptor agonist buspirone or of the SSRI paroxetine accelerates the occurrence of fatigue of human subjects performing prolonged exercises [59,60].

It is therefore a paradox that central fatigue seems to involve spinal motoneurons [61]. How can 5-HT promote the excitability of motoneurons and at the same time induce central fatigue? This question was recently investigated [16••]. It appears that moderate synaptic release of 5-HT produced by stimulation of the raphe spinal pathway promotes the excitability of motoneurons by activating 5-HT_2 receptors located in synapses covering the somato-dendritic compartments (Figure 2b) [62]. By contrast, a prolonged release of 5-HT mimicking what happens during intense motor activity [19], decreases the excitability of motoneurons by activating extrasynaptic 5-HT_{1A} receptors expressed at the axon initial segment (AIS) of motoneurons [16••]. A detailed analysis involving electrophysiology, pharmacology, immunohistochemistry and cyclic voltammetry, showed that prolonged stimulation of the raphe spinal pathway induces spillover of 5-HT and allows the neurotransmitter to reach the AIS, which is devoid of serotonergic synapses [16••,63]. There, the activation of 5-HT_{1A} receptors inhibits Na^+ channels responsible for the genesis of action potentials in motoneurons (Figure 2c). Consequently the firing threshold is increased, the gain of motoneurons is reduced and central fatigue occurs. Under these conditions, motoneurons respond to a given synaptic input by generating fewer action potentials and thereby trigger a weaker muscle contraction. The function of central fatigue has been debated. Because it occurs during intense motor activity, one of its roles could be to prevent too strong contraction that would be damaging for the muscle [54]. In addition, experiments performed on human subjects clearly show that central fatigue also occurs during weak voluntary muscle contractions [64]. The resulting increase in the firing threshold of motoneurons ensures the rotation of motor units occurring during any sustained movement [65,66••]. We postulate that the spillover of 5-HT is responsible for the decrease of the excitability of active motoneurons. If this mechanism accounts for rotations, it implies that the release of 5-HT is stronger on active motoneurons than on silent ones. However, local mechanisms regulating the release of 5-HT remain to be found.

Figure 2



5-HT exerts a dual modulation of motoneurons. **(a)** A motoneuron responds to an input (blue) by action potentials that propagate along the axon (green bars). This triggers the contraction of the muscle fibers it innervates and generates a force (F). **(b)** Moderate release of 5-HT activates intrasynaptic receptors that promote L-type Ca^{2+} channels and inhibit leak K^+ channels. The excitability is increased and the input will induce more action potentials in the motoneuron. Consequently the force generated is stronger. (c) During intense release of 5-HT a spillover occurs. 5-HT reaches extrasynaptic receptors located at the AIS. This inhibits Na^+ channels responsible for the genesis of action potentials. Fewer spikes are generated and the muscle force is decreased. This mechanism is responsible for central fatigue.

What is the origin of central fatigue and muscle rotation in humans? Is inhibition of action potential initiation in motoneurons by 5-HT involved? To test this hypothesis one should estimate the excitability of motoneurons during motor tasks and modify the activation of 5-HT receptors. It is possible to determine the excitability of motoneurons by measuring F-waves evoked by supramaximal stimulation of a peripheral motor nerve [67•]. Comparing the occurrence of F-waves before and after prolonged muscle contraction would tell if fatigue is concomitant with a change in motoneuron excitability. Repeating the same experiment after oral ingestion of 5-HT_{1A} receptor agonists/antagonists or of SSRIs could indicate if 5-HT is responsible for the change in excitability.

Future challenges and opportunities

In summary, it seems that during low-level of motor activities, moderate release of 5-HT exerts excitatory effects on movement genesis, while during higher level, stronger release inhibits both locomotion and motoneuron excitability.

Crucial data is missing for full understanding of how 5-HT contributes to motor control. One should determine the precise subcellular distribution of serotonergic receptors in the spinal cord. The presynaptic, intrasynaptic and extrasynaptic distribution of 5-HT receptors and their presence in presynaptic and postsynaptic neuronal compartments is crucial information still missing. This is a real challenge that awaits new tools. The available antibodies directed against 5-HT_{1A} receptors give strikingly different stainings. For example, an antibody developed on the second extracellular loop of the receptor provides a consistent staining of the AIS of neurons [68], while two different antibodies directed against the third intracellular loop either label neuronal somata [69] or somatodendritic compartments [70]. It should be noted that none of these antibodies has been tested on knockout animals. Another challenge is to activate the raphe spinal pathway in a controlled manner. So far this has been attempted with localized stimulus electrodes [35,62]. The advent of optogenetics should provide new approaches with increased selectivity and precision. The recent development of mice that express channelrhodopsin in raphe nuclei allows the selective activation of serotonergic neurons by blue light [71•,72••]. These models could be extremely useful for testing how different release levels of 5-HT modulate pattern generation and motoneuron excitability.

Conflict of interest statement

Nothing declared.

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- •• of outstanding interest

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