Neuromodulation of Vertebrate Locomotor Control Networks

Vertebrate locomotion must be adaptable in light of changing environmental, organismal, and developmental demands. Much of the underlying flexibility in the output of central pattern generating (CPG) networks of the spinal cord and brain stem is endowed by neuromodulation. This review provides a synthesis of current knowledge on the way that various neuromodulators modify the properties of and connections between CPG neurons to sculpt CPG network output during locomotion.

The neural networks that control locomotion in vertebrates, so called central pattern generators (CPGs), are located primarily in the spinal cord. CPGs produce a basic rhythmic motor output in the absence of sensory feedback, largely from the interplay between the electrical properties of the constituent neurons and the nature of the synaptic interconnections between them. However, these components of CPGs are subject to neuromodulation deriving from a wide range of sources both intrinsic to the spinal cord and also projecting to the spinal cord from other, extrinsic sources. The neuromodulators involved are chemically diverse, ranging from simple amino acids to biogenic amines, peptides, and even a gas, nitric oxide. For the most part, these neuromodulators act on G-protein-coupled receptors to alter the concentration of intracellular second messengers. The response properties of individual CPG neurons can be dramatically modified in the presence of any one of this range of neuromodulators. Growing evidence points to the existence of complex interactions between different modulatory inputs to the motor system. In effect, the prevailing “cocktail” of neuromodulators endows locomotor CPGs with an almost infinite range of output configurations. This is necessary to enable the short term flexibility and long term adaptability required of locomotion during the life time of the individual.

The precision, grace, and elegance with which animals navigate smoothly and efficiently through their environment ultimately results from a complex set of interactions between the individual neural components, the CPG networks that drive rhythmic firing in motoneuron, the biomechanical properties of peripheral propulsive structures, and the integration of sensory information with central motor control networks. The ability of a neural system to adapt in the face of external demands is often essential for survival, and this ability in turn requires that it generates a motor output that is inherently flexible. Neuromodulators represent an important source of this flexibility at the level of the underlying neuronal networks of the central nervous system and the motor output they produce. Here, we review current evidence on the sources and effects of the various neuromodulators that influence vertebrate locomotor control networks and provide an account of how they achieve their modulatory influences.

Role of Neuromodulation in Modifying Centrally Generated Activity

The rhythmic firing patterns of motoneuron that underpin locomotory movements such as walking, swimming, or flying, result from the activity and inter-connections of premotor interneurons that form complex networks residing in the spinal cord and brain stem (reviewed in Ref. 65). Neuromodulators change locomotor activity via one or both of two routes: modulation of the integrative electrical properties of motoneuron and CPG interneurons, and modulation of the synaptic connections between interneurons and motoneuron. As a broad rule of thumb, it is possible to infer the locus of a neuromodulators influence on the locomotor output by determining whether it affects the frequency and/or the amplitude of the output. The hypothetical locomotor rhythm shown in FIGURE 1 illustrates motor nerve recordings from functionally antagonistic motoneuron pools in which the pattern of bursting activity strictly alternates during rhythm generation.

If the frequency of the rhythm stays the same in the presence of a neuromodulator but the amplitude or duration of each motor burst changes, then the effects are most likely occurring predominantly at the level of the motoneuron (or last order interneurons; Bi in FIGURE 1). Alternatively, if the burst properties...
are unaffected but the frequency is altered, then the neuromodulator is most likely affecting rhythm-generating circuitry without major influences on downstream neurons (Bii in FIGURE 1). In many cases, both amplitude and frequency change, which suggests that the neuromodulator affects motoneurons and CPG neurons simultaneously (Biii in FIGURE 1).

Conceptually, there are two possible sources of neuromodulator that can affect locomotor CPGs (89): extrinsic sources such as modulatory inputs that descend from the brain and that affect CPGs remotely, and intrinsic modulators that are released from cells that are embedded within the CPGs (89). In both cases, neuromodulators usually exert their effects by acting as agonists at metabotropic receptors, some of which increase excitability, whereas others decrease excitability.

Opposing Effects of Neuromodulators on Sensory vs. Motor Systems

For monoamines, 5-hydroxytryptamine (5-HT) in particular, there is a strong inverse correlation between their effects on motor output and sensory input: if motor output is facilitated, then sensory inputs are diminished and vice versa. This seems logical because at times when an increase in locomotor activity is necessary, a down-tuning of sensory transmission will help to ensure that innocuous extrinsic sensory inputs do not interfere with ongoing locomotor activity. How are these opposing effects accomplished? The most likely explanation for this functional dichotomy is that the receptors to which 5-HT is coupled are expressed differentially in sensory and motor circuits such that receptors in one pathway are positively coupled to a second messenger and are negatively linked to the same second messenger in the other pathway and vice versa. In addition, monoamines differentially modulate ascending sensory information relating to locomotor movements (68). However, this review focuses on neuromodulation of motor systems, and effects on sensory systems will not be discussed further.

Role of Neuromodulation Developmental Network Adaptation

Some of the neuromodulators that exert acute modulation of locomotor activity also play important

FIGURE 1. Schematic showing rhythmic, alternating motor output generated by antagonistic motor pools
Schematic showing how rhythmic, alternating motor output generated by antagonistic motor pools (A) can be altered in amplitude (i), frequency (ii), or both (iii), depending on whether a given modulator acts at the level of the motoneurons (Bi), the CPG (Bii), or a combination of the two (Biii).
roles in the longer term during motor system development. 5-HT, for example, has been shown in a variety of vertebrate motor systems to have a major influence on circuit assembly during early development, and, in keeping with this role, the fibers that invade the spinal cord from the brain stem arrive there at the critical points in circuit maturation when major changes in the motor output are taking place. In chick embryos, ablation of serotoninergic projections in ovo using neurotoxins dramatically affects synaptogenesis of inputs to spinal motoneuron (130a). In Xenopus frog tadpoles, application of a 5-HT neurotoxin or 5-HT receptor antagonist during embryogenesis prevents the normal postembryonic maturation of swimming activity, suggesting that descending raphe-spinal projections are causal to locomotor network development (149). In rodents, as in lower vertebrates, descending projections of the raphe system also invade the spinal cord at critical stages in network assembly, and 5-HT profoundly modulates locomotor activity, inferring a prominent role for the amine in development (168).

**Evolution of 5-HT Signaling**

5-HT is a phylogenetically ancient signaling molecule, distributed throughout the metazoans, which plays key roles in the development and modulation of locomotor function in vertebrates and invertebrates alike. In chordates like the lancelet, immunocytochemical evidence reveals serotonergic neurons in the dorsal cerebral vesicle and in the spinal cord arranged in a ladder-like ventral chain close to the central canal (23). Lampreys belong to a primitive vertebrate group, the agnathans, which split away from the main vertebrate line around 500 million years ago. Lampreys possess an intrinsic modulatory plexus running as a strip spanning the ventral midline, which serves as an important source of 5-HT (183), dopamine (156), and other modulators like substance P.

**5-HT in Mammalian Systems**

Almost all 5-HT in the mammalian spinal cord originates from brain stem raphe nuclei and the parapyramidal region (reviewed in Ref. 99). A range of spinal neurons relevant to locomotor control in mammals receive serotonergic input, as identified by their close association with 5-HT-labeled synaptic boutons. These neuronal targets for 5-HT include spinal motoneurons (3), Renshaw cells (26), commissural interneurons of laminae VII and VIII (67), V0C interneurons (182), V2-derived interneurons (2), Hb9 interneurons (178), and neurons, predominantly in laminae VII and VIII, shown to be activated during locomotion via c-fos expression (128). There is also widespread expression of 5-HT receptors in the mammalian spinal cord, with 5-HT<sub>2</sub> receptors dominating in the ventral horn (reviewed in Ref. 144).

5-HT can both activate and modulate mammalian locomotor networks. Activation of spinal locomotor networks by 5-HT was first shown in the rabbit using the 5-HT precursor 5-HTP (167). 5-HT was later shown to also initiate locomotor activity in isolated spinal cord preparations obtained from neonatal rats and mice (27, 36, 92, 112, 127) and spinalized adult rats and mice (55, 100, 165). In contrast, 5-HT appears insufficient to initiate locomotion in the spinalized cat (6, 7). However, 5-HT is not without effect on cat locomotion. In spinalized cats, activation of 5-HT receptors leads to an increase in the amplitude and duration of bursts of on-going locomotor-related EMG activity recorded from hindlimb muscles (6, 7). In addition, in cats recovering from incomplete spinal transections, 5-HT increases the regularity of stepping (21). 5-HT also modulates on-going locomotor activity in rodents with the effects varying depending on the receptor subtypes activated. The effects of 5-HT<sub>2</sub> and 5-HT<sub>7</sub> receptor activation are generally

**Sources and Types of Neuromodulator**

The neuromodulators that have been shown to affect vertebrate locomotion fall into three main categories: the biogenic amines (5-HT, noradrenaline, dopamine, and trace amines), amino acids normally acting on metabotropic receptors (GABA<sub>µ</sub>, mGluRs), and peptides (e.g., substance P). In addition, many other molecules that do not fit neatly into these categories have also been shown to alter locomotor activity including the purines (ATP and adenosine), d-serine, endocannabinoids, and nitric oxide. In the following sections, we summarize evidence on the sources and effects of these neuromodulators across a range of vertebrate locomotor networks.

**Biogenic Amines**

The sources of the amines depend partly on the species of vertebrate in question. Homologous groups of aminergic neurons are located in the brain stem of most if not all species, including the raphe nuclei (5-HT), the locus coeruleus (noradrenaline), and the substantia nigra (dopamine). Additional groups include in mammals the serotonergic parapyramidal region (PPR) of the brain stem and the A11 dopaminergic nucleus of the dorsoposterior hypothalamus. In lower vertebrates, notably the lamprey, there are additional sources of amines located in the spinal cord itself (see below).
facilitatory causing increases in the frequency and/or amplitude of locomotor activity recorded from in vitro preparations (14, 46, 62, 108). In addition, activation of 5-HT_3_ and 5-HT_7_ receptors strengthens left/right and flexor/extensor alternation during fictive locomotion (107, 132). In contrast, the activation of 5-HT_7_ receptors has “inhibitory” effects slowing the frequency of locomotor activity (25, 46). Taken together, these data indicate that 5-HT can modulate motoneurons or last-order interneurons to affect the magnitude of the final output of the locomotor network and interneurons involved in rhythm generation to affect the timing and coordination of locomotor activity.

Neuromodulators affecting motoneurons often target persistent inward currents (PICs). PICs can amplify synaptic inputs and give rise to plateau potentials that allow for periods of sustained firing (reviewed in Ref. 74). PICs and their associated plateau potentials are enhanced by 5-HT receptor activation in the cat (37, 81), rat (15, 73, 106), and turtle (82, 134). This enhancement appears to involve the facilitation of Na<sup>+</sup> and Ca<sup>2+</sup> PICs along with a reduction in K<sup>+</sup> currents (16, 73, 82, 106, 134). Thus direct or indirect modulation of PICs in motoneurons is likely to contribute to the effects of 5-HT on the amplitude of locomotor output from spinal networks. Other modulatory effects of 5-HT on motoneuronal properties that may contribute include a reduction in the voltage sensitivity of NMDA channels and subsequent facilitation of NMDA receptor-dependent oscillations (110, 111) and modulation of inwardly rectifying K<sup>+</sup> channels and Ih currents (94).

In addition to modulating motoneurons, 5-HT modulates the intrinsic properties of spinal interneurons via several mechanisms that may underlie the effects of 5-HT on the frequency of locomotor activity. The most common effect of 5-HT on spinal interneurons is to depolarize their resting membrane potential (24, 40, 184–186). In addition, 5-HT decreases the action potential threshold in the majority of commissural interneurons (184, 185), an undefined population of ventral horn interneurons (52), and interneurons that are active during locomotion (40). Interestingly, 5-HT has also been shown to compress the range of voltage-thresholds for repetitive firing in ventral horn interneurons by reducing firing thresholds in high threshold interneurons and increasing firing thresholds in low threshold interneurons (162). Other effects of 5-HT on interneurons include a reduction in the magnitude of the AHP in most commissural interneurons (45, 184, 185) and locomotor activity-related interneurons (40), as well as modulation of Ih currents and enhancement of PICs in locomotor activity-related interneurons (41, 42). Although 5-HT has a range of modulatory effects on the intrinsic properties of spinal interneurons, the common consequence of this modulation is neuronal excitation. This suggests that excitation of either inhibitory or excitatory CPG neurons, depending on the receptor subtype involved, may underlie the mixed facilitatory and inhibitory actions of 5-HT on locomotor activity. Alternatively, novel inhibitory actions of 5-HT on spinal interneurons may remain to be discovered.

Finally, besides affecting locomotion via the modulation of intrinsic properties of spinal neurons, 5-HT may also exert its effects by modulating synaptic transmission. Evidence for 5-HT-mediated modulation of synaptic transmission in the ventral spinal cord includes the modulation of sensory and descending inputs to spinal interneurons in the cat (67, 69, 86) and sensory input to motoneurons in the rat (11).

**Noradrenaline in Mammalian Systems**

Noradrenergic input to the mammalian spinal cord originates from A5, A6 (locus coeruleus), and A7 brain stem nuclei (34, 130, 174). Examples of mammalian spinal neurons innervated by these brain stem sources of noradrenalin (NA) include motoneurons (61, 79, 138), commissural interneurons (67), and lamina VII premotor interneurons (116). There is also widespread expression of adrenergic receptors in the mammalian spinal cord (60, 129, 142, 143).

Like 5-HT, NA can both initiate locomotor activity and modulate ongoing locomotor patterns. In spinalized cats, activation of noradrenergic, particularly α2, receptors powerfully stimulates locomotor activity (7, 31, 56, 91). In contrast to the cat, NA induces only poor locomotor-like activity in isolated rat spinal cord preparations (57, 93, 154). In both chronic spinalized cats and isolated rat spinal cord preparations, application of NA during ongoing locomotor activity slows the rhythm (31, 93) and increases the amplitude of EMG or ventral root activity (31, 93, 154). By using specific agonists, it can be shown that the modulation of locomotor activity by NA reflects the net effect of separate mechanisms activated by different adrenoceptor subtypes. During locomotion in chronic spinal cats, α2-adrenoceptor activation increases step cycle duration, primarily by lengthening burst duration in flexor muscles. In contrast, activation of α1-adrenoceptors has little effect on the timing of locomotion but instead increases the strength of extensor muscle activity (31). These data suggest that α2-adrenoceptor activation influences interneurons involved in rhythm generation, whereas α1-adrenoceptor activation modulates motoneuron output. Data from rodent studies demonstrate a similar separation in the effects...
of $\alpha_1$- and $\alpha_2$-adrenoceptor activation. In isolated rat spinal cord preparations, activation of $\alpha_2$-adrenoceptors leads to a decrease in the frequency of pharmacologically induced locomotor activity with no effect on the amplitude of ventral root bursts. In contrast, activation of $\alpha_1$-adrenoceptors results in an increase in locomotor frequency and a decrease in the amplitude of ventral root bursts (154). Interestingly, $\alpha_1$-adrenoceptor activation reduces locomotor frequency and increases ventral root burst amplitude when locomotion is induced by stimulation of the cauda-equina in isolated mouse spinal cord preparations (62). This may reflect species differences or indicate that the mode of activation of locomotion influences the potential modulatory roles of NA.

The dominant effect of NA on spinal neurons seems to be an increase in excitability, most likely via activation of $\alpha_1$-adrenoceptors, whereas activation of $\alpha_2$-adrenoceptors can inhibit activity (51, 131, 161, 154, 176). NA shares many cellular mechanisms of neuromodulation with 5-HT. In parallel with 5-HT, NA activation can facilitate persistent inward currents and plateau potentials (35, 104, 162), reduce inwardly rectifying $K^+$ currents (161), hyperpolarize the action potential threshold (52), and compress the range of voltage-thresholds for repetitive firing in ventral horn interneurons (162). However, unlike 5-HT, NA appears to have little modulatory effect on the AHP (161).

Adrenoceptor activation, like serotonergic receptor activation, may also alter locomotor activity by modulating synaptic connectivity with the spinal cord. One of the most striking examples of this comes from experiments utilizing the isolated rat spinal cord in which the application of NA un-masks recurrent excitatory pathways that can modulate locomotor activity, increasing the frequency of rhythmic activity (109). NA also increases locomotor-related synaptic drive to motoneurons in the isolated rat spinal cord (161), an effect that may contribute to the increased motor output seen upon adrenoceptor activation. Finally, like 5-HT, NA can modulate sensory and descending inputs to spinal interneurons in the cat (67, 69, 86) and sensory input to motoneurons in the rat (11).

**Dopamine in Mammalian Systems**

In mammals, dopaminergic inputs, arising predominantly from the hypothalamic A11 region (18, 78, 137, 150), are distributed throughout the ventral horn of the spinal cord (80, 172, 181). In addition, all subtypes of dopamine receptors (D1–D5) are also expressed within the ventral horn (187, 188).

Involvement of dopaminergic input in locomotor control is supported by measurements of increased dopamine levels in the spinal cord during locomotor activity (59). However, dopamine is not as effective as 5-HT or NA at initiating locomotion. In spinal cats, dopaminergic agonists fail to induce locomotion (7). In contrast, in mice with spinal cord transections, locomotion can be induced by D1/D5 receptor agonists (102), suggesting that specific activation of D1/D5 receptor subtypes might induce locomotor activity in the cat. In isolated rodent preparations, the ability of dopamine receptor activation to induce locomotion varies by species. In neonatal rat preparations, dopamine can induce rhythmic locomotor-like activity, although it is much slower than that induced by 5-HT and in some reports more irregular (10, 92). In contrast, in neonatal mouse preparations, the activation of dopamine receptors alone is insufficient to induce locomotor activity, but activation of dopamine receptors (primarily D1 and D2) by endogenous dopamine is required for 5-HT-induced locomotor activity (112). Interestingly, at later developmental time points in mice, toward 1 wk old and beyond, exogenous dopamine is required along with 5-HT and NMDA to elicit locomotor activity in isolated spinal cord preparations (86a, 124).

In both cat and rodents, dopamine can modulate ongoing locomotor activity. In the cat, dopamine receptor activation increases the amplitude of flexor activity during locomotion (7), primarily suggesting modulation of motoneurons. In comparison, in isolated rodent spinal cord preparations, dopamine receptor activation slows locomotor rhythms initiated by 5-HT and NMDA while also increasing the amplitude of locomotor-related output and making the activity more reliable (10, 62, 175). Thus, in rodents, dopamine is likely to modulate the activity of interneurons involved in rhythm generation and motoneurons.

Analyses of the mechanisms by which dopamine receptor activation modulates mammalian spinal neurons are at present limited to motoneurons and Hb9$^+$ interneurons in isolated neonatal mouse spinal cord preparations (70, 71). In mouse motoneurons, dopamine application increases excitability by modulating potassium currents including $Ca^{2+}$-dependent $K^+$ currents to reduce the mAHP and $I_K$ to reduce the latency to the first spike (70). In addition, activation of the D1 subtype of dopamine receptors modulates AMPA receptor-mediated currents in motoneurons (71). In the case of Hb9$^+$ interneurons, dopamine application has been shown to be necessary but not sufficient to induce oscillatory behavior, which may relate to the locomotor rhythm (77, 178). In addition to modulating the intrinsic properties of motoneurons and Hb9$^+$ interneurons, dopamine modulates synaptic input to motoneurons including those from sensory afferents (11, 25, 33) and may modulate...
Biogenic Amines in Non-Mammalian Systems

In contrast to studies of mammalian systems, research involving aquatic vertebrates has demonstrated that amimergic signaling pathways are functionally integrated, often providing complementary modulatory effects (118). The modulatory effects of biogenic amines in these animals will therefore be discussed together.

In the lamprey, the modulatory effects of 5-HT have been a topic of intense research and debate. Bath applied 5-HT has a well defined effect on locomotor activity; the rhythm slows and the bursts intensify in a similar manner to other vertebrates. The fact that the 5-HT uptake inhibitor citalopram has a similar effect to 5-HT indicates that endogenous release of the amine can regulate burst formation during locomotion. The mechanisms of action of 5-HT classically involve a reduction of the slow after-hyperpolarization (sAHP) that follows the action potential in motoneuron mediated in large part by apamin-sensitive Ca$^{2+}$-dependent K$^+$ (K$_{Ca}$) channels (reviewed in Ref. 65a). The sAHP acts as a brake on motoneuron discharge by determining the time it takes for the neuron to recover and fire again. Hence, in reducing the sAHP, 5-HT reduces spike accommodation, and so, for a given excitatory input, motoneuron fire for longer and at a higher frequency. The serotonergic block of K$_{Ca}$ therefore neatly explains the increase in discharge frequency of motoneurons within each cycle of swimming. Modulation of the sAHP in CPG interneurons can also affect their firing frequency and consequently change the frequency of the locomotor rhythm. The same K$_{Ca}$ channels also contribute to the plateau phase of the intrinsic NMDA receptor-mediated membrane potential oscillations reported in lamprey motoneurons when they are activated by the calcium entry that follows NMDA receptor activation (169a). K$_{Ca}$ channel activation drags the membrane potential from the depolarized plateau phase into a sufficiently more hyperpolarized region so that Mg$^{2+}$ ions can block the open NMDA receptor ion channel and trigger the falling phase of the oscillation. The serotonergic block of K$_{Ca}$ channels therefore prolongs the depolarized plateau phase, allowing neurons to fire for longer in each cycle. Thus, through parallel actions of 5-HT on K$_{Ca}$ channels and NMDA receptors on spinal neurons, 5-HT modulates frequency, duration, and amplitude of locomotor bursts.

Given that K$_{Ca}$ channels play a major role in the modulation of lamprey locomotion by 5-HT, it would be anticipated that apamin, which blocks K$_{Ca}$ channels, should have the same effects as 5-HT on swimming. However, even at high concentrations (10 µM) sufficient to block the sAHP and increase motoneuron firing, apamin had no significant effect on the frequency of NMDA-induced fictive locomotion (123a). This result can only be partially reconciled by the fact that the effects depend on the initial concentration of NMDA and the starting frequencies of swimming that this elicits. Since 5-HT has also been shown to reduce the strength of excitatory synaptic transmission via a presynaptic mechanism (145), a more complete explanation is that, to mediate its full effects on locomotion, 5-HT must have additional effects besides blocking K$_{Ca}$ channels.

In zebrafish, the effects of 5-HT are developmentally stage specific. At early stages, 5-HT modulates the duration of quiescent periods, and thus the frequency of swim bouts, without directly affecting the firing of neurons active during swimming or the various parameters of a swim cycle (19). This appears to be mediated by an effect on chloride homeostasis (20). Once the zebrafish becomes free swimming, and continuing into adulthood (58), 5-HT has effects that parallel those in the lamprey, namely the cycle period lengthens. In this case, however, the mechanism appears to involve a strengthening of the mid-cycle glycinergic inhibition, akin to the effect of NA in Xenopus tadpoles (118). In Xenopus tadpoles, 5-HT applied to young larvae increases the duration of motor bursts but has little effect on the locomotor frequency, producing a relatively fast, short, and intense version of the fictive swimming rhythm. In contrast, NA has the opposite effect of increasing the cycle period but has little or no effect on the duration of motor bursts. Which receptor subtypes are involved in the serotonergic modulation of locomotion? In both lamprey (76) and Xenopus tadpoles, there is evidence that 5-HT1a receptors are involved in some of the effects, and for the lamprey it has been reported that the effects of K$_{Ca}$ channels are largely mediated by this receptor and its negative coupling to adenylate cyclase (AC) (177). Also in the lamprey, it has been reported that the serotonergic presynaptic inhibition of excitatory transmission is mediated by the 5-HT1d receptor (145), which also negatively couples AC. In mammalian systems, the receptor subtypes differ in that endogenous release of the amine can regulate burst formation during locomotion. The mechanisms of action of 5-HT classically involve a reduction of the slow after-hyperpolarization (sAHP) that follows the action potential in motoneuron mediated in large part by apamin-sensitive Ca$^{2+}$-dependent K$^+$ (K$_{Ca}$) channels (reviewed in Ref. 65a). The sAHP acts as a brake on motoneuron discharge by determining the time it takes for the neuron to recover and fire again. Hence, in reducing the sAHP, 5-HT reduces spike accommodation, and so, for a given excitatory input, motoneuron fire for longer and at a higher frequency. The serotonergic block of K$_{Ca}$ therefore neatly explains the increase in discharge frequency of motoneurons within each cycle of swimming. Modulation of the sAHP in CPG interneurons can also affect their firing frequency and consequently change the frequency of the locomotor rhythm. The same K$_{Ca}$ channels also contribute to the plateau phase of the intrinsic NMDA receptor-mediated membrane potential oscillations reported in lamprey motoneurons when they are activated by the calcium entry that follows NMDA receptor activation (169a). K$_{Ca}$ channel activation drags the membrane potential from the depolarized plateau phase into a sufficiently more hyperpolarized region so that Mg$^{2+}$ ions can block the open NMDA receptor ion channel and trigger the falling phase of the oscillation. The serotonergic block of K$_{Ca}$ channels therefore prolongs the depolarized plateau phase, allowing neurons to fire for longer in each cycle. Thus, through parallel actions of 5-HT on K$_{Ca}$ channels and NMDA receptors on spinal neurons, 5-HT modulates frequency, duration, and amplitude of locomotor bursts.

Given that K$_{Ca}$ channels play a major role in the modulation of lamprey locomotion by 5-HT, it
bouts. Dopamine, or the dopamine uptake inhibitor bupropion, abolishes swimming activity, an effect mediated in the brain stem. Dopamine has no effect on the integrative electrical properties of spinal neurons. Pharmacological blockade of D2 receptors or activation of adenylate cyclase (a downstream target that is inhibited by D2 receptors) blocks the inhibitory effect of dopamine. The suppression of swim initiation appears to be transient since, by 5 days postfertilization, dopamine uptake no longer affects the frequency of swim episodes.

**Conventional Fast Excitatory Transmitters**

Conventional transmitters involved in the fast synaptic interactions that occur during locomotor network operation mediate their effects by activating ionotropic postsynaptic receptors (glutamate: NMDA, AMPA; GABA: GABAa; acetylcholine: nAChR; glycine: glyR). However, the same transmitters (with the possible exception of glycine) also activate metabotropic receptors located both pre- and postsynaptically to modulate ongoing activity. In many cases (e.g., group 2 mGluRs, mAChR, GABAa), the presynaptic receptors mediate homosynaptic depression of transmitter release and thus function as negative feedback autoreceptors. In certain cases, pharmacologically similar receptors are located on the terminals of adjacent neurons where they can mediate heterosynaptic depression or facilitation of transmitter release. However, other metabotropic receptors for classically fast transmitters are located postsynaptically, such as mGluR1 receptors, where a range of complex modulatory effects have been described.

**Glutamate (mGluRs)**

Glutamate is best known for its role as the major fast, excitatory neurotransmitter in the CNS, a role it fulfills via the activation of ionotropic glutamate receptors. However, glutamate can also act as a neuromodulator by binding to three groups (I–III) of G-protein-coupled metabotropic glutamate receptors (5, 136). Given that the functioning of the locomotor CPG is dependent on glutamatergic signaling between spinal interneurons (13, 160, 175), there are considerable intraspinal sources of glutamate. In addition, the locomotor CPG receives glutamatergic input from both descending (66, 88) and sensory systems (117). In addition to the abundance of glutamatergic transmission affecting spinal motor circuitry, there is widespread expression of mGluRs in the ventral horn (4, 103) that could be activated in parallel with ionotropic glutamate receptors (44).

The effects of metabotropic glutamate receptors (mGluRs) within vertebrate motor systems have been most extensively studied in the lamprey (49, 95) (FIGURE 2), with comparative data arising from the tadpole (28, 29) model system. In aquatic vertebrates, as in other species, group 2 and 3 mGluRs appear to be located primarily presynaptically where they mediate presynaptic inhibition of glutamate release and therefore function as negative feedback controllers of excitatory transmission. Activation of these receptors generally slows fictive locomotion. In contrast, group 1 mGluRs play a variety of different, mainly excitatory, roles, and in both lamprey and tadpole their activation leads to an increase in the locomotor frequency. In both species, there is evidence that this effect is due partly to postsynaptic modulation of neuronal excitability and partly to presynaptic inhibition of glycinergic inhibitory transmission. Group 1 mGluRs can be further subdivided into mGluR1 and mGluR5 subtypes. In the lamprey spinal cord, mGluR1 (but not mGluR5) receptors cause membrane potential depolarization and excitation of spinal cord neurons by blocking a leak (mixed Na+/K+) current.

The effect requires activation of phospholipase C (PLC) and the release of calcium ions from intracellular stores. mGluR1s also enhance excitability of lamprey spinal neurons by facilitating current flow through NMDA receptors that play a key role in the rhythmic excitation that occurs in each cycle of locomotion. In parallel, mGluR5 receptors regulate lamprey locomotion but via different mechanisms that trigger oscillations in intracellular calcium and have a net inhibitory effect on the frequency of locomotion (90). In contrast, in frog tadpoles, both subtypes of group 1 mGluRs increase locomotor frequency (29). Experiments using mGluR1- and mGluR5-specific antagonists demonstrate that endogenous activation of both types of receptors contribute to intrinsic spinal modulation of locomotor output in lamprey and frog tadpoles (29, 90, 95). These data on both young frog tadpoles and adult lampreys also infer a conserved mGluR-mediated modulatory mechanism that is established early in vertebrate development (tadpole) and evolution (lamprey), namely the presynaptic inhibition of glycine release by group 1 mGluRs. Although this conserved modulatory mechanism suggests a strategic positioning of group 1 mGluRs on the synaptic terminals of inhibitory interneurons of the spinal CPG, there is extensive evidence from the lamprey system that activation of group 1 mGluRs couples to an intracellular signaling cascade that triggers the release of the endocannabinoid 2-AG from postsynaptic membranes that activates presynaptic endocannabinoid receptors to reduce inhibitory transmission.
This pathway is reviewed in detail in a recent issue of this journal (50).

Activation of mGluRs also modulates, but does not initiate, locomotor activity in isolated rodent spinal cord preparations (85, 157–159). Analyses have concentrated on the role of group I mGluRs, although group II and III mGluRs also modulate rat locomotor activity (159). In the rat spinal cord, the application of group I mGluR agonists either disrupts locomotor activity completely or slows it (158). Seemingly paradoxically, group I-specific mGluR antagonists also decrease the frequency of locomotor activity (158). The authors of these studies suggest that this apparent discrepancy reflects diffuse excitatory effects of exogenous agonist overshadowing more specific depressant effects of endogenous group I mGluR activation. In contrast to these data in the rat but consistent with findings in lamprey (95) and Xenopus tadpoles (29), recent data obtained from isolated mouse spinal cord preparations have demonstrated an increase in locomotor activity on activation of group I mGluRs (85). In addition to affecting the frequency of locomotion, group I mGluR activation also modulates the amplitude of locomotor-related motoneuron output in mice, indicating modulatory effects at the level of CPG interneurons and motoneurons.

**FIGURE 2.** Activation of mGluR1 receptors increases locomotor frequency in the lamprey by divergent neuromodulatory effects

Glutamate released from excitatory interneurons in the spinal cord activates locomotor network neurons via ionotropic AMPA and NMDA receptors and simultaneously activates postsynaptic mGluR1 receptors. mGluR1s block a leak K⁺ channel, enhance NMDA receptor-mediated currents, and trigger release of the endocannabinoids, which bind to EC receptors on glycinergic interneurons to reduce mid-cycle inhibition. Figure was adapted from Ref. 50 and used with permission.
Data concerning the cellular effects of mGluRs on mammalian spinal neurons are limited to the effects of group I mGluR activation on motoneurons (85, 114, 115). Application of group I mGluR agonists leads to depolarization of motoneuronal resting membrane potentials in both rats and mice. In rat motoneurons, this is associated with reports of no change (115) or an increase in resistance (114), whereas in mouse motoneurons mGluR-dependent depolarization is associated with a decrease in resistance (85). In mouse motoneurons, group I mGluR activation also reduces the amplitude of transient Na⁺ currents, an effect that seems to lead to reduced motoneuron firing and decreased locomotor-related motoneuron output (85). In both rats and mice, group I mGluRs also modulate synaptic transmission within the spinal cord. In rats, group I mGluRs appear to depress inhibitory transmission involved in sensorimotor pathways (115) and recurrent inhibitory transmission to motoneurons (114). In mice, activation of group I mGluRs depresses excitatory locomotor-related input (85).

**Acetylcholine**

All cholinergic inputs to mammalian spinal neurons are thought to originate from within the spinal cord (119, 147, 166). Motoneurons represent the major acetylcholine-producing cells of the spinal cord. The acetylcholine they produce is mostly destined for the periphery where it mediates transmission at the neuromuscular junction, although motoneuron axon collaterals also activate recurrent inhibitory pathways mediated by Renshaw cells (38, 98). Other intraspinal sources of acetylcholine include small cholinergic neurons scattered in the dorsal horn, central canal cluster cells surrounding the central canal, and “partition cells” that lie between the dorsal and ventral horns in a region extending from lamina X to the lateral edge of the gray matter (8, 83, 124, 135, 182). Along with neuronal sources of acetylcholine, there is considerable expression of muscarinic metabotropic acetylcholine receptors in the ventral horn of the mammalian spinal cord (75, 125, 171, 173, 179). Taken together with the fact that cholinergic interneurons are activated during locomotion (83), these data highlight the potential importance of acetylcholine as a modulator of locomotor behavior.

In the early embryonic mouse spinal cord, cholinergic transmission, arising from the axon collaterals of motoneurons and involving nicotinic receptors, drives early locomotor-related rhythm and appears to contribute to the appropriate assembly of spinal locomotor circuitry (72, 126). Later, at neonatal stages in the rat, locomotor-like activity can be initiated by activation of metabotropic receptors following the application of acetylcholine or cholinesterase inhibitors (36, 151, 152). It should, however, be noted that this acetylcholine-driven activity often lacks appropriate alternation between ipsilateral extensors and flexors (36).

Although there have been no reports of acetylcholine modulating the frequency of ongoing locomotor activity in mammals, cholinergic transmission at C-bouton synapses, which densely cover motoneuronal somata and arise from spinal interneurons (V0c interneurons), has recently been shown to modulate the strength of locomotor-related motoneuron output in a task-dependent manner (124, 182) (FIGURE 3). Spinal V0c interneurons, which are the source of C-bouton inputs to motoneurons, appear to be tonically active throughout locomotion with the frequency of their activity tightly phase locked to motoneuron output. Genetic inactivation of the output of V0c interneurons results in an impaired ability to increase the activation of hindlimb muscles during motor tasks that require greater force. From these data, it is predicted that task-specific modulation of the intensity of motoneuron output is in part controlled by V0c interneurons and their C-bouton contacts with motoneurons.

At the cellular level, acetylcholine-mediated modulation generally has a net excitatory effect on spinal neurons. Studies in mice have shown that acetylcholine depolarizes the resting membrane potential and decreases input resistance in both commissural interneurons and interneurons that are activated during locomotion (which may include some commissural interneurons) (24, 40). Additional effects of acetylcholine observed in locomotor activity-related interneurons include a hyperpolarization of the action potential threshold, an increase in the magnitude of the AHP, and a decrease in Ih (40, 41). The net excitatory effect of acetylcholine on mouse interneurons is evidenced by a leftward shift in frequency current relationships (24), although in locomotor activity-related interneurons this is accompanied by a decrease in slope such that excitability is only increased at lower stimulus intensities (40).

In mouse motoneurons, activation of metabotropic muscarinic receptors also has a net excitatory effect on firing output (124). Although muscarinic receptor activation can either hyperpolarize or depolarize the resting membrane potential of motoneurons, it always leads to a reduction in AHP amplitude via the activation of m2-type muscarinic receptors (124), which are found at C-bouton synapses (75, 125, 179) (FIGURE 3). This reduction in the AHP leads to increased motoneuron excitability as evidenced by an increase in the slope of frequency current plots (124) and is thought to underlie the task-dependent regulation.
of the strength of motoneuron output during locomotion discussed above (182).

For lower vertebrates, much less is currently known about the role of metabotropic muscarinic receptors for acetylcholine in the modulation of locomotor activity. However, there is ample evidence that motoneurons express muscarinic receptors. In the salamander, for example, M2-type receptor activation alters the integrative electrical properties of motoneurons by modulating three ionic currents: a hyperpolarization-activated cationic current (I_h), a calcium-dependent potassium current (I_{Ca}), and an inwardly rectifying potassium current (I_{KIR}) (32).

**Conventional Fast Inhibitory Transmitters**

**GABA/Glycine**

There is an abundance of inhibitory interneurons within the mammalian spinal cord, which, in addition to controlling key aspects of the locomotor pattern such as the alternation of activity between...
the left and right sides of the spinal cord and between flexor and extensor motoneurons, may also modulate on-going locomotor activity. Commonly studied examples of inhibitory ventral interneurons include Ia inhibitory interneurons (53, 84), Renshaw cells (141), and inhibitory commissural interneurons (e.g., Refs. 22, 101). Along with the presence of many sources of inhibitory transmission within the spinal cord, there is also widespread expression of metabotropic GABA\(_B\) receptors (30, 164), supporting a potential role for neuromodulation of locomotion via inhibitory transmission.

In the mammalian spinal cord, a number of studies in cats (e.g., Refs. 39, 48, 87, 105, 155) and rodents (17, 170) have shown that the main modulatory actions of GABA\(_B\) receptors involve the presynaptic inhibition of transmitter release with little or no effect on postsynaptic properties of neurons. In isolated neonatal rat spinal cord preparations, the consequences of GABA\(_B\) receptor activation on locomotor output include a reduction in the amplitude of ventral root bursts, probably due to reduced locomotor-related synaptic drive to motoneurons, and a reduction in locomotor frequency due to undefined actions on CPG interneurons (17).

With respect to GABA\(_B\) receptors in lower vertebrates, it has been shown that their activation in Xenopus embryos also causes presynaptic inhibition of transmitter release, specifically from the terminals of glycinergeric interneurons that mediate reciprocal inhibition during swimming (169). The effect involves a direct presynaptic action on omega-conotoxin-sensitive (N-type) calcium channels, leading to a reduction in the probability of quantal release. In addition, a GABA\(_B\) receptor agonist reduces the firing threshold of motoneurons, indicating parallel pre- and postsynaptic effects that unite to reduce the duration and frequency of fictive swimming. Similar effects of GABA\(_B\) receptor activation have been reported in the lamprey where tonic activation of these receptors during fictive swimming contributes to the setting of the locomotor burst frequency. In addition, GABA\(_B\) receptors play an important role in controlling the intersegmental propagation of activity along the spinal cord during fictive locomotion (115a, 161a). Activation of GABA\(_B\) receptors by endogenously released GABA during locomotor network activity reduces the locomotor frequency and modifies the intersegmental phase lag as the activity propagates rostrocaudally along the spinal cord (161a). These effects are mediated in large part by the GABA\(_B\)-mediated depression of voltage-activated calcium currents and Ca\(^{2+}\)-dependent K\(^+\) channels, which affects post-inhibitory rebound and the post-spike after hyperpolarization (115a).

**Purinergic Transmitters**

In Xenopus embryos, ATP and adenosine control the duration of swimming episodes (43). The sources of ATP are not identified, but it is presumed to be co-released by neurons that are part of the spinal CPG. At the onset of a swimming bout, extracellular ATP levels rise as a result of the activity. Initially, near the onset of the bout when swim frequency is at its highest, ATP blocks K\(^+\) channels to help maintain a high level of excitability within the network so the swimming frequency is at its highest. However, as the episode progresses, the ATP is gradually broken down in the extracellular space by a 5’-ectonucleotidase to adenosine. Adenosine is able to block Ca\(^{2+}\) channels, which impairs synaptic transmission and reduces excitability. Evidence suggests that adenosine mediates its effects via A1 receptors and that both an N-type calcium current and a further unidentified HVA calcium current are inhibited by adenosine (18b). The effect of adenosine’s inhibition of calcium channels is to lower the swim frequency, reducing the activity-dependent production of ATP by the swim CPG. With time, the inhibitory effects of adenosine overcome the excitatory effects of ATP, and the swim frequency eventually reaches a critical low level that can no longer be sustained, and thus the swimming bout ceases. This is a very elegant example of a role for an intrinsic neuromodulator that may be important in ensuring that swimming does not continue ad infinitum. There are more traditional mechanisms for terminating swimming such as inhibitory reticulospinal neurons that produce rapid GABAergic inhibition whenever the rostral cement gland is contacted by objects in the environment (18a). In behavioral terms, this descending inhibition from the brain stem is likely to be involved before the purinergic biochemical clock mechanism is engaged.

Recent work utilizing isolated neonatal mouse spinal cord preparations has also revealed a role for purines in the intrinsic modulation of the mammalian locomotor CPG (180). In the mouse, spinal cord adenosine, derived from the breakdown of ATP following its release from glia, appears to play an endogenous role in setting the excitability and frequency of the locomotor CPG. This is revealed by an increase in locomotor frequency upon blockade of adenosine receptors that is absent in the presence of glial toxins or ectonucleotidase inhibitors.

**Peptide Neuromodulators**

The best understood peptide with respect to roles in motor control is the tachykinin, substance P. In
lower vertebrates, it has been extensively studied in the isolated spinal cord of the lamprey where a brief (10 min) application of substance P leads to both short-term (~2 h) and long-term (>24 h) increases in the frequency of NMDA-induced fictive locomotion (131a, 131b). The short-term effects are protein kinase C-dependent and appear to involve a potentiation of cellular responses to NMDA and the associated induction of membrane potential oscillations. The long-term effects, in which a 10-min exposure to substance P triggers an increase in the baseline swimming frequency that lasts for up to 24 h, require changes in protein synthesis. The tachykinin receptor antagonist spantide II leads to a lowering of the baseline NMDA-induced locomotor frequency, strongly suggesting that the release of substance P or a related peptide during swimming modulates the locomotor CPG. Substance P also has a presynaptic facilitatory effect on the release of glutamate onto spinal neurons via a calcium-independent mechanism (Parker & Grillner, 1998). There is an intrinsic source of tachykinins in the spinal cord: substance P immunoreactivity is found in cells of the ventral plexus. However, there is also evidence for two tachykinins (substance P and neurokinin B) in numerous cell groups in the brain, including regions that project to the spinal cord and may play a role in the initiation and/or modulation of swimming. It is not known under what circumstances the lamprey might engage this positive feedback system to maintain high-frequency swimming, but since these animals undergo long migrations to their breeding grounds it has been hypothesized that the tachykinin system may be well suited to contributing to such a behavioral role.

In mammals, a large number of peptides and their associated receptors are present in the ventral aspect of the spinal cord and are therefore in a position to modulate motor activity (12, 133). In one study (9), 11 such peptides were applied to the isolated rat spinal cord, either on their own or in the presence of NMA or NMA plus 5-HT, a cocktail of drugs that activates the locomotor CPG, while recordings were made from lumbar ventral roots. Only four of the peptides (oxytocin, vasopressin, bombesin, and TRH) were able to trigger tonic or loosely coordinated rhythmic activity when bath applied alone. However, all of the peptides were able to modulate ongoing locomotor cocktail-induced CPG activity, although the effects were different for each peptide. For example, proctolin and neurotensin increased the frequency of locomotion, whereas met-enkephalin and oxytocin decreased it. Some peptides (bombesin, somatostatin) acted purely on the frequency of locomotion, whereas most affected both the rhythm frequency and the locomotor burst amplitudes. Presumably, the peptides acting solely on amplitude can directly influence motoneuron firing, whereas the others also act at the level of premotor interneuronal circuitry.

Gaseous Neuromodulation

The free radical nitric oxide (NO) is an unusual but potent modulator of locomotor activity in lower vertebrates such as the Xenopus tadpole (120, 122) and the lamprey (96, 97). NO is manufactured by cells that possess the enzyme NO synthase, which breaks down L-arginine to L-citrulline, liberating a molecule of NO in the process. In Xenopus tadpoles, the neuronal sources of NO, as revealed by NADPH-diaphorase histochemistry, are located in the brain stem in distinct and bilaterally symmetrical neuronal clusters (120, 123). NOS expression in these neurons appears in a well defined temporal sequence such that all three clusters are labeled around the time of hatching from the egg membrane when the young larva is only ~3 days old. These three clusters remain throughout development (139). NO has a net inhibitory effect and acts as a break on swimming: NO donors reduce episode durations and slow swimming; NOS inhibitors or NO scavengers have the opposite effect, indicating that NOS is constitutively active and produces an intrinsic NO tone that regulates swimming output (120). The primary mechanisms through which these effects on locomotion occur include NO’s presynaptic facilitation of 1) glycinergic transmission (which slows the swimming rhythm) and 2) GABAergic transmission (which leads to premature termination of activity by activating a descending “stopping” pathway mediated by reticulospinal inhibitory interneurons) (121, 122) (FIGURE 4). At these early stages of development, before the onset of free swimming, NO is inhibitory. However, later in larval development, around the time when the limbs are developing, NOS-positive neurons appear in the spinal cord (139), and NO donors have the opposite effect of facilitating rhythmic activity (148).

Although there is extensive anatomical evidence that NO synthesis is widespread in the nervous system of most vertebrates studied, its potential role in the modulation of locomotion in other model systems has received considerably less attention than other more conventional signaling molecules. Recent studies have shown that NO plays a significant role in modulating locomotion in both the lamprey (96, 97) and mouse (1, 47).

In the lamprey system, NOS is located in a variety of spinal neuron types including proprioceptive edge cells and gray matter neurons. NO
has an excitatory effect, increasing the frequency of NMDA-induced locomotor activity. NOS inhibition or NO scavenging has the opposite effect, showing that there is an intrinsic release of NO during locomotor rhythm generation. The synaptic effects of NO in the lamprey are complex. NO simultaneously reduces mid-cycle (glycinergic) inhibition and potentiates on-cycle (glutamatergic) excitation. Analysis of miniature synaptic currents reveals a purely presynaptic effect on glycine release but a parallel pre- and postsynaptic effect on glutamatergic transmission.

Spinal interneurons expressing NOS are also found throughout the mouse spinal cord. Although NOS-positive interneurons are most abundant in the dorsal horn, they are also present at lower levels in areas surrounding the central canal and parts of the ventral horn, particularly lamina VII (47, 124, 153). The addition of NO donors to isolated mouse spinal cord preparations modulates both the amplitude and frequency of on-going locomotor activity (47). Interestingly, the amplitude of locomotor activity can either be increased or decreased, depending on the dose of the NO donor, whereas frequency is consistently reduced. NO scavengers also affect the amplitude and frequency of locomotor output, indicating an endogenous role for NO in modulating the activity of both CPG interneurons and motoneurons.

**Metamodulation and Interactions Between Modulators**

In some notable cases, the effects of 5-HT and NA are opposing and can switch during development (118, 140). 5-HT, in general, facilitates the locomotor drive, increasing the duration and intensity of ventral root bursts. In *Xenopus* tadpoles, NA exerts effectively the opposite effects by lengthening the cycle period without any obvious effect on the duration of motor bursts. How are these differential effects produced? The two amines have a common target in modulating the strength of glycinergic inhibition (118). Both amines act presynaptically to regulate glycine release from commissural interneurons, but whereas 5-HT depresses release, NA enhances it. In the case of 5-HT, the effect is to reduce the amplitude of the inhibition at mid-cycle, allowing neurons to escape from inhibition sooner and fire for longer. In the case of NA, the effect is to increase the amplitude of mid-cycle inhibition and delay the onset of the next cycle.

Later in development, during the metamorphic period when the limbs and associated central circuits are being assembled, the roles of 5-HT and NA, although still acting in opposition, switch. Rauscent and colleagues studied the effects of two biogenic amines, 5-HT and NA, on the spontaneously generated fictive locomotor patterns recorded from ventral roots at this later stage (140). It is first shown that these isolated preparations display one of two forms of circuit coordination: either the limb and tail circuits generate rhythms with independent frequencies or they generate a conjoint rhythm with 1:1 coordination. Following the addition of 5-HT to preparations displaying the frequency-independent rhythms, the slower limb rhythm accelerates while the faster axial rhythm slows until the two are united at a single common frequency. Conversely, in preparations already dis-

---

**FIGURE 4.** Modulation and metamodulation of the *Xenopus* tadpole locomotor network

Summary diagram illustrating the metamodulation of spinal inhibition by NO in *Xenopus* tadpoles. At larval stage 42 (top), the major subdivisions of the CNS (middle) are recognizable, including the forebrain (fb), midbrain (mb), hindbrain (hb), and spinal cord (sc). Brain regions are separated by dotted lines. The noradrenergic locus coeruleus (in orange) is located just caudal to the midbrain-forebrain boundary. The mid-hindbrain reticulospinal (Mhr) region (purple) is located dorsally in the caudal hindbrain, with axons that cross the midline (dashed line) and descend to the spinal cord, providing a major source of spinal GABAergic inhibition. Commisural interneurons (blue) cross the midline and provide the major source of spinal glycinergic inhibition. NO modulates episode durations by directly modulating GABA release (blue) to motoneurons (MN). NO modulates swimming frequency by altering endogenous NA release (orange), which in turn modulates glycine release (blue) to motoneurons (MN). Basal levels of NO are indicated in middle circle, reduced NO levels in left circle (pale colors), raised NO levels in right circle (dark colors). Figure adapted from Ref. 121, and used with permission from *Journal of Neuroscience*. [1201] 10.220.32.246 on April 27, 2017 http://physiologyonline.physiology.org/ Downloaded from
playing the frequency-coupled rhythm, bath applications of NA exerts the opposite effect of decelerating the limb and accelerating the axial rhythm until the two display independent frequencies. These effects are similar to those exerted by the amines at stages both before and after metamorphosis; 5-HT decelerates the axial rhythm of earlier stages before limb development, whereas NA slows (while 5-HT accelerates) the limb rhythm generated by froglets after the tail has disappeared. At the intermediate stages when both circuits are present, it is presumably important for the organism to be able to functionally couple and decouple the two locomotor systems, and Rauscent and colleagues suggest that two aminergic systems with opposing behavioral effects may be involved in this tactical switching between two locomotory networks.

Metamodulation defines a second-order form of modulation in which the effects of one modulatory system are influenced by the effects of another modulatory system (89). Metamodulation can occur in series or in parallel. A clear example of serial metamodulation in vertebrate locomotor control involving NO signaling is to be found in the swimming system of *Xenopus* tadpoles (121) (FIGURE 4). The differential effects of the amines 5-HT and NA on inhibitory transmission have already been described (see above). In the case of NA, there is a presynaptic facilitation of glycine release from commissural inhibitory interneurons and a parallel presynaptic facilitation of GABA release from descending midhindbrain reticulospinal interneurons. The effects of NO bear a remarkable similarity, raising the question of whether these two modulators have convergent targets at the terminals of GABAergic and glycineergic interneurons or whether there might be serial interactions between the two modulatory systems. Considering first the modulation of GABA synapses, there is an apparent convergence along two parallel routes: one a nitrergic effect and the other noradrenergic. The evidence is that the effects of a given neuromodulator on GABA transmission is unaffected by blockade of the other modulator and vice versa. For example, the shortening of swim episode durations (due to enhanced GABA release) by NA persists when NO is scavenged or NOS is inhibited, and the effects of NO donors on episode durations remain when alpha-adrenergic receptors are blocked.

In the case of glycineergic transmission, however, the situation is very different because the effects of NO are occluded when alpha-adrenergic receptors are blocked by phentolamine, but the effects of noradrenaline are unaffected by NO scavenging or NOS inhibition. This leads to the conclusion that NO’s effects on glycine transmission involve a facilitation of the noradrenergic system, which in turn mediates the direct presynaptic facilitation of glycine release.

Conclusions and Future Prospects

Vertebrate locomotor control networks are richly endowed with neuromodulatory inputs that provide the locomotor output with the degree of flexibility that is necessary to maneuver efficiently through the environment. These inputs derive from multiple locations both within the spinal cord and also from extrinsic modulatory centers in the brain. At the cellular level, we know most about modulators deriving from neuronal sources, but it is clear that, even though we appreciate them less, glia are able to synthesize and release neuromodulators with very potent effects on central circuitry, including ATP, D-serine, and neurosteroids.

In aquatic vertebrates, such as the lamprey and frog tadpole, the links between cellular mechanisms of action and their consequences for locomotor network output are well described for a number of neuromodulators. In comparison, the relationships between cellular mechanisms and network output are less clear for the neuromodulation of complex mammalian systems. This largely reflects our lack of knowledge regarding the components of the mammalian CPG and their interconnectivity. However, with an increasing number of mammalian interneurons being defined and described using molecular genetic techniques (63), we are now much better placed to go on to define specific sources of neuromodulation, their cellular targets, and their roles in the regulation of mammalian locomotor output (e.g., Ref. 182).

Although knowledge of locomotor circuitry is critical toward understanding the actions of neuromodulators, conversely, studies of neuromodulation may aid in deciphering locomotor networks. This has indeed proved to be the case for brain stem networks controlling mammalian respiration where knowledge of substance P and opioid-mediated modulation of the respiratory rhythm led to the discovery that NK-1 and mu-opioid receptors serve as markers of critical respiratory rhythm-generating neurons within the pre-Botzinger complex (64). More recently, the specific neuromodulatory effects of opioids on pre-Botzinger complex neurons have also revealed the existence of a second respiratory oscillator in the brain stem, the parafacial respiratory group (reviewed by Ref. 54).

From the perspective of the individual components comprising locomotor control networks (i.e., neurons and synapses), it is abundantly clear that they are constantly exposed to an ever changing neuromodulatory environment. At any moment in time, the extent to which the plethora of modulatory receptors embedded in the neuronal mem-
brane are activated will determine the levels of the intracellular second messenger molecules that ultimately determine the computational ability of the neuron to integrate fast synaptic inputs and generate output. In this sense, the neurons can be thought of as biochemical integrators, a role that ought to be given equal credence with their role of transmitting electrical information to their own synaptic targets. This idea is by no means a new one (89), and yet in the last decade there has been very little progress in advancing the hypothesis. This is surely an area in which future research effort should advance our understanding of how neuromodulation sculpts an infinite variety of outputs from a single anatomically defined network.

No conflicts of interest, financial or otherwise, are declared by the author(s).

References


