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Spinal-Cord Plasticity

Independent and Interactive Effects of Neuromodulator and Activity-Dependent Plasticity

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Abstract

Plasticity is one of the most extensively studied aspects in neuroscience. Interest in it has primarily been related to its proposed role in learning and memory and its relevance to adaptive changes following injury. Plasticity can be evoked by changes in molecular, cellular, and synaptic properties, either as a result of activity-dependent effects, or by relatively slow-acting neuromodulatory transmitters. In addition, it is increasingly recognized that the plasticity evoked by these individual effects can be altered by previous inputs and is thus itself plastic. Here, I will review studies in the lamprey spinal cord that have examined individual and interactive activity-dependent and neuromodulator-mediated plasticity. The results show that activity-dependent and neuromodulator-mediated plasticity evoke neuron-and synapse-specific effects at different levels in the spinal cord, and that interactions within and between these effects can evoke dynamic changes in cellular, synaptic, and network plasticity.

Index Entries: Metaplasticity; activity-dependent synaptic plasticity; neuromodulation; neuropeptide; spinal cord; interactive plasticity; interneuron; neural network.

Introduction

In order to understand how behavior is generated by the nervous system, we need to know the functional properties of the neural networks that are used to process sensory inputs, perform cognitive functions, or program motor outputs. Network activity arises from the integration of molecular, cellular, and synaptic properties. While these properties must be able to generate specific network outputs, they must also be flexible to allow plasticity of the output in response to changes in internal or external environments. There are thus two related questions: how do molecular, cellular, and synaptic properties interact to evoke network activity, and how are these properties modulated to allow short- or long-term plasticity of the network output?

Plasticity can be evoked by activity-dependent cellular or synaptic changes *(see 1–3)*, or by the modulation of cellular and synaptic

properties through G protein-coupled transmitter receptors *(see 4–5).* These are relevant mechanisms in their own right, and to facilitate their analysis they have largely been studied independently. In vivo, however, interactions can occur within and between these effects. Thus, synaptic activity can influence subsequent activity-dependent synaptic plasticity *(6);* neuronal activity can influence neuromodulator release *(7);* neuromodulator interactions can evoke additive, inhibitory, or novel modulatory effects *(8,9);* and neuromodulators can influence the expression of activity-dependent synaptic plasticity *(10,11).* Although the functional relevance of these interactions is not well-understood, the complexity or subtlety that could result is daunting. Cellular and synaptic properties will not simply be plastic, but depending on previous or subsequent inputs the plasticity could itself be either plastic or modulatory. "Stable" states may thus reflect a dynamic equilibrium between interacting effects, or the point at which these effects have been interrupted, for example during developmental *(see 5,12),* circadian *(13,14),* or seasonal variations *(15,16).*

The Use of the Lamprey as a Model System

In order to examine plasticity, information is required on the basic output of the system under study. My analysis has used the lamprey spinal cord as a model system in which to examine individual and interactive plasticity. The lamprey spinal locomotor network generates a basic output that is simple both to monitor and to analyze. Although far from complete (*see* below), information is also available to some extent on the cellular and synaptic mechanisms that generate network activity, and also on sensory and descending inputs to the spinal cord.

The lamprey is a cyclostome, a lower vertebrate, which swims using undulatory movements of its elongated body. These movements are generated by pattern generating networks in each of the approx 100 spinal-cord segments that in association with descending and sensory inputs coordinate alternating contractions of the myotomes on the left and right sides of the body. The range of aspects examined in the lamprey demonstrates its general utility in examining nervous-system function *(17–22).* Several features make it an attractive model system. Firstly, the spinal cord lacks a blood supply. It is instead oxygenated directly from the cerebrospinal fluid (CSF), thus allowing the intact spinal cord to be isolated and maintained in oxygenated Ringer in vitro. Secondly, behaviorally relevant locomotor-network activity can be evoked in the isolated spinal cord (i.e., in the absence of sensory or descending inputs) by bath-applied excitatory amino acids. Finally, cellular and synaptic properties can be examined in the intact spinal cord by recording from physiologically identified neuronal classes *(23).* Together, these features allow cellular and synaptic properties to be examined in an intact functional network in vitro. Thus, providing that identified neurons and synapses are used, some of the uncertainties associated with extrapolating between neurons and levels in dynamic and nonlinear physiological systems *(24,25)* can to some extent be avoided.

Spinal plasticity can occur at three levels: directly on the locomotor network, on its descending brainstem inputs, or on its sensory inputs. Plasticity at these levels will interact in vivo, effects at any level potentially influencing the final motor output. Cutaneous sensory inputs can be examined by recording from mechanosensory afferents (dorsal cells) and identified sensory interneurons *(26),* and descending inputs by recording from reticulospinal axons and their postsynaptic spinal targets (*27; see* Fig. 1A). Locomotor-network effects can be examined by investigating the plasticity of N-methyl-D-aspartate (NMDA)-evoked network activity.

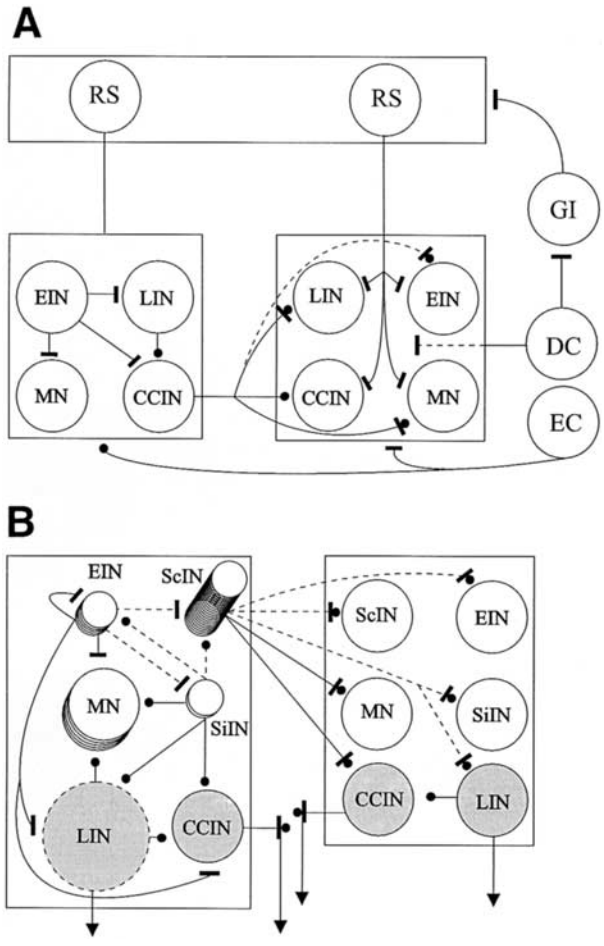

Fig. 1. The segmental locomotor network in lamprey. **(A)** Segmental locomotor networks on both sides of the spinal cord, and their sensory (DC, mechanosensory dorsal cell; EC, edge cell; GI, giant spinobulbar interneuron) and descending inputs (RS, reticulospinal neuron). Symbols represent populations of neurons. Inputs shown are from populations. It is not known as yet to what extent single cells diverge. This segmental network consists of motor neurons (MN), which are assumed to be pure output elements (but *see* ref. *108*); excitatory network interneurons (EIN), which provide ipsilateral glutamatergic excitation; glycinergic crossed caudal interneurons (CCIN), which provide contralateral inhibition between networks on both sides of the spinal cord; and lateral interneurons (LIN), which provide ipsilateral glycinergic inhibition to CCINs. Uncertainties with this segmental network (*see* text) have recently led to an alternative scheme **(B).** This essentially places the LIN and CCINs (shaded in this and subsequent figures) as intersegmental interneurons, and replaces them at the segmental level with small ipsilateral inhibitory interneurons (SiIN) and small crossing interneurons (ScIN; *29;* see text). In the left panel in (B), the approximate relative sizes and numbers of each type of interneuron are shown. Each circle represents groups of 10 interneurons in each hemisegment (with the exception of LINs; there is usually one LIN per segment in the rostral region of the cord). In both networks, circles represent inhibitory glycinergic inputs, and bars excitatory glutamatergic inputs. Mixed symbols represent interneuron classes that can be either inhibitory or excitatory. In (A) and (B), ipsilateral connections are shown on the left, and contralateral, sensory, and descending connections on the right. Dashed lines indicate connections for which evidence is currently lacking.

The Lamprey Locomotor Network

The cellular and synaptic basis of network plasticity can be examined by recording from identified network neurons. A model of the lamprey locomotor network was suggested by Buchanan and Grillner (*28; see* Fig. 1A for details). Uncertainty still exists, however, over the identification and organization of network components. Rovainen *(29)* proposed that the lateral and crossing interneurons (LIN and CCINs, respectively; *see* Fig. 1A) were not segmental interneurons, but that they acted intersegmentally to coordinate activity along the spinal cord. For the LINs this was because their long axonal projections were inconsistent with a segmental role, and also that they are only found in rostral regions of the spinal cord. For the CCINs, reasons included that they cannot solely account for contralateral inhibition *(30),* and that stimulating a single CCIN did not affect segmental network activity. Although single interneurons may not be expected to exert significant effects, the limited number (6–14 per segment) and connectivity *(30,31)* of the CCINs suggests that stimulation of a single cell should have an effect if they play a significant segmental role. Finally, CCIN

axons project at least 14 segments caudally *(30).* This is also inconsistent with a segmental role; recent results from Buchanan *(32)* suggest that segmental reciprocal inhibitory inputs extend only 5 segments caudally. LINs and ipsilateral inhibition were removed from certain network models *(33).* Recent physiological studies suggest that LINs are not important for segmental network activity *(34),* supporting their proposed intersegmental role *(29).*

Two classes of potential network interneurons were identified after the initial network model was proposed. The first are small ipsilateral inhibitory interneurons (SiIN; *35*). These are rhythmically active during network activity, and stimulation of a single SiIN significantly affects ongoing network activity. Ipsilateral inhibition may thus be required in segmental network simulations *(36).* The second interneuron class is a large population (~50% of the neurons per segment) of contralaterally projecting interneurons (ScIN; *37*). Little information is available on these cells. They resemble CCINs in that they project to contralateral networks, but they have smaller cell bodies and, significantly in relation to the findings of Buchanan (*32; see* above), axons that project only up to five segments caudally.

The SiINs and ScINs may be the segmental homologs of the LIN and CCINs originally suggested by Rovainen *(29).* As with the CCINs, ScINs can be inhibitory or excitatory *(23).* Preliminary data suggests that these interneurons evoke significantly larger PSPs (~ 4mV) than other spinal interneurons (~ 1 mV), and that stimulation of inhibitory or excitatory ScINs can affect ongoing cellular and network activity (Parker, unpublished observations). A proportion of the excitatory ScINs may be sensory interneurons, because they can receive monosynaptic inputs from sensory dorsal cells, and do not usually exhibit rhythmic membrane potential oscillations during network activity.

Uncertainty thus exists over the organization of the locomotor network, a not-untypical problem in network analyses *(38).* The initial analysis of network plasticity included CCIN and LINs (*see* Fig 1A). Due to difficulties with this segmental model, including those described earlier, the current network analysis uses a tentative model that includes SiINs and ScINs as segmental ipsilateral and contralateral inhibitory interneurons, respectively, with CCIN and LINs acting as intersegmental interneurons (*see* Fig. 1B).

This review is divided into four sections: activity-dependent plasticity and metaplasticity, neuropeptide-mediated spinal modulation, long-term neuropeptide-mediated network plasticity, and the interactive effects of neuromodulators.

Activity-Dependent Plasticity of Network Interneuron Synaptic Transmission

Rhythmic network neurons repetitively fire single or bursts of spikes *(39).* As a result activity-dependent synaptic plasticity was suggested as a potential mechanism for patterning rhythmic activity *(40).* Little attention, however, has focused on its role (but *see 41,42*).

Activity-dependent synaptic plasticity was examined in the lamprey locomotor network by making paired recordings from identified classes of network neurons *(31,43,44).* Stimulation trains that mimicked interneuron spiking during network activity (i.e., up to five spikes at frequencies of 5–30 Hz; *23*) could evoke activity-dependent synaptic plasticity (*see* Fig. 2). Plasticity was specific to the class of interneuron examined, and within a single class was dependent on the postsynaptic target and the stimulation frequency (*see* Fig. 2 and Table 1). These features are exemplified by the depression of EIN-evoked inputs. The frequency-dependence of depression was 5 Hz = 10 Hz > 20 Hz in motor neurons, 20 Hz = 10 Hz > 5 Hz in CCINs, and 5 Hz = 10 Hz = 20 Hz in excitatory interneuron (EINs).

Several aspects of this plasticity could be relevant to the patterning of network activity. Accumulative homosynaptic depression could act as an endogenous burst-terminating mechanism by regulating synaptic strengths during

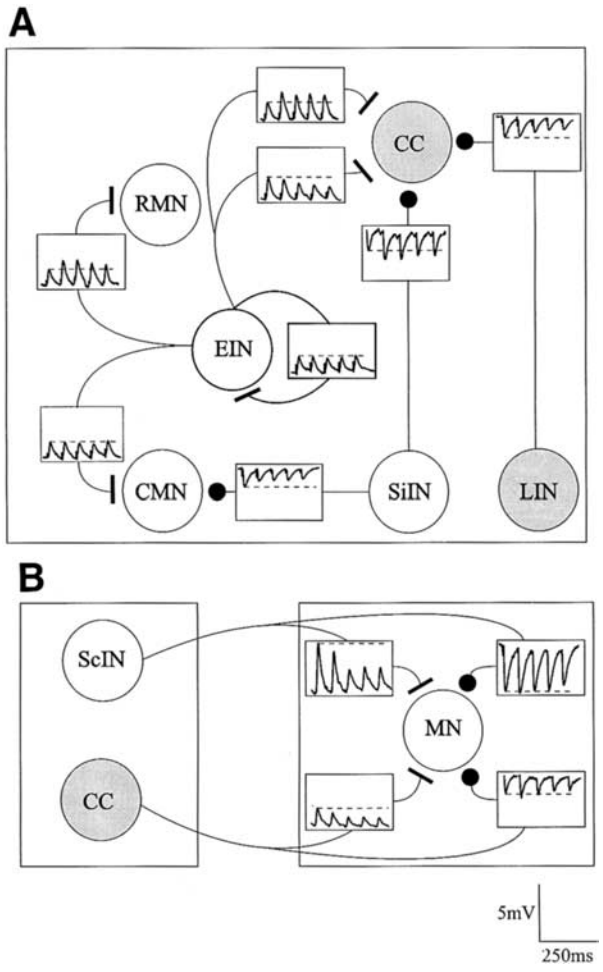

Fig. 2. Summary of the activity-dependent plasticity of ipsilateral **(A)** and contralateral **(B)** network interneuron synaptic inputs. RMN and CMN refer to motor neurons that are rostral or caudal to EINs, respectively. Other symbols and abbreviations are as in Fig. 1. Traces in the boxes show typical examples of the plasticity evoked by a 20 Hz spike train over five spikes. Note that whereas most inputs are equivalent in size, those from the ScINs are typically larger. Horizontal dashed lines on this and subsequent figures extend from the peak of the initial PSP to illustrate plasticity during the train.

locomotor activity. For example, the depression of EIN inputs could reduce synaptic drive and terminate ipsilateral activity, whereas the depression of crossed inhibitory inputs could reduce the inhibition of contralateral networks, and thus allow them to become active. Facilitation could also contribute. For example, the facilitation of inhibitory SiIN inputs to the crossed inhibitory CCINs could act as a heterosynaptic burst-regulating factor by disinhibiting contralateral networks. Depression and facilitation mechanisms could also act synergistically. The depression of EIN inputs and the facilitation of inhibitory SiIN inputs to CCINs will reduce ipsilateral excitation but enhance ipsilateral inhibition, respectively, to synergistically reduce crossed inhibition and allow the contralateral network to become active. Spatial aspects of the plasticity could also contribute. Although inputs from EINs rostral to motor neurons did not usually exhibit significant plasticity over bursts of five spikes at physiologically relevant frequencies, inputs from EINs located caudal to motor neurons facilitated. This result supports the suggestion of Cohen et al. *(45)* that intersegmental co-ordination may depend on functionally stronger ascending synaptic inputs.

Synaptic properties in the spinal cord thus vary widely (*see* Fig. 2 and Table 1). This is a typical finding, and can also include variability in the output of a single neuron onto two or more different postsynaptic targets *(46).* It is not yet known whether the variability of synaptic properties in the lamprey reflects different divergent outputs from single interneurons, or differences in populations of neurons. The specific plasticity of divergent inputs from a single neuron to different postsynaptic targets will allow a single presynaptic pattern of action potentials to have multiple parallel representations in postsynaptic targets. However, divergent outputs from EINs appear to be rare *(31),* suggesting that differences in synaptic inputs may instead reflect variability in interneuron populations. This suggests a difference in organization properties between descending brainstem neurons, which do diverge *(31),* and spinal locomotor networks, and between interneurons in spinal networks and those in cortical and hippocampal neurons *(46,47).* These differences presumably reflect the specific functional requirements of the systems.

Table 1
Summary of the Activity-Dependent Plasticity of Network Interneuron Synaptic Transmission[a]

Cell type	Input	Effect	Region (and frequency-dependence) where significant effects initially occur	Frequency-dependence of plateau plasticity	Effect during spike bursts
EIN-caudal MN	EPSP	Depress	$Train_{6–10}$ (5 Hz, 10 Hz)	5 Hz = 10 Hz > 20 Hz	Constant
EIN-rostral MN	EPSP	Facilitate	$Train_{1–5}$ (5–20 Hz)	20 Hz > 10 Hz > 5 Hz	?
EIN-EIN	EPSP	Depress	$Train_{6–10}$ (5–20 Hz)	20 Hz = 10 Hz = 5 Hz	?
EIN-CCIN	EPSP	Depress	$Train_{1–5}$ (5–20 Hz)	20 Hz = 10 Hz > 5 Hz	Depress
EIN-CCIN	EPSP	Facilitate	$Train_{1–5}$ (5–20 Hz)	20 Hz = 10 Hz = 5 Hz	Depress
SiIN-CCIN	IPSP	Facilitate	$Train_{1–5}$ (20 Hz)	20 Hz > 10 Hz = 5 Hz	?
SiIN-MN	IPSP	Depress	$Train_{1–5}$ (10 Hz)	10 Hz > 20 Hz > 5 Hz	Constant
LIN-CCIN	IPSP	Depress	$Train_{1–5}$ (5 Hz, 10 Hz)	20 Hz = 10 Hz = 5 Hz	?
ScIN-MN	IPSP	No change	–	–	Facilitate
ScIN-MN	EPSP	Depress	$Train_{1–5}$ (5–20 Hz)	20 Hz = 10 Hz = 5 Hz	Depress
CCIN-MN	IPSP	Depress	$Train_{6–10}$ (5, 10 Hz)	5 Hz = 10 Hz > 20 Hz	?
CCIN-MN	EPSP	Depress	$Train_{1–5}$ (5 Hz, 10 Hz)	5 Hz = 10 Hz > 20 Hz	Depress

[a] The columns show the connection examined, the plasticity evoked, the region and frequency over which a significant effect was initially evoked, and the frequency dependence of the plateau level of plasticity at the different frequencies examined. Effects occurring over the first five spikes ($Train_{1–5}$) are of relevance to interneuron inputs during network activity *(23)*. Notice that linear frequency-dependent effects were uncommon. Notice also that connections have mainly been examined between interneurons and motor neurons. Several features of the plasticity that cannot be summarized in a single table and thus the original papers should be consulted for further details *(31,42,43)*. The repeating of connections reflects variability in responses.

Buchanan *(48)* has shown that cellular properties also vary within single classes of interneurons. Variability of spinal cellular and synaptic properties is thus a general theme. Sub-populations with different cellular and synaptic properties presumably exist within single classes of interneuron that can be recruited by sensory or descending inputs to generate flexible motor outputs. This potential variability in interneuron populations is not reflected in network diagrams, or presumably in network activity evoked by the indiscriminate pharmacological activation of all network components.

The Regularity of Synaptic Inputs During Network Activity

A specific problem faced by interneurons in rhythmic networks is that they can be active for hours, days, or, in the case of respiratory networks, a lifetime. Mechanisms must thus exist to ensure that synapses generate consistent and reliable responses during different patterns of network activity. This does not mean that plasticity cannot occur. As shown earlier, it is evoked by stimulation trains that mimic interneuron spiking during network activity. What is important is that the synaptic response during spike bursts does not change significantly over time. Although these changes may be relevant under certain conditions, for example, successive depression over spike bursts acting to terminate network activity, they must be avoided if sustained, reliable network activity is required.

The regularity of network synaptic transmission was investigated by examining the properties of EIN inputs to motor neurons *(43)*. EINs were examined because they are the only interneurons whose proposed segmental network role has not been questioned. Motor neurons were used as postsynaptic targets as they

are simple to record from and identify. EIN inputs to motor neurons depressed over a single train of 20 spikes at 5–20 Hz. The depression was caused by transmitter depletion that was opposed by an ongoing activity and calcium-dependent mechanism, possibly related to the replenishment of releasable transmitter stores *(49).* The equilibrium between depletion and replenishment mechanisms will presumably determine the plateau level of depression. Although depression occurred over a single train of 20 spikes, repetitive bursts that mimicked interneuron spiking during network activity (i.e., 5 spikes at 5–20 Hz) did not depress, even when up to 2,500 excitatory postsynaptic potentials (EPSPs) were evoked *(43).* This reliability was activity-dependent. In addition, synaptic transmission over repeated bursts, but not single low-frequency-evoked EPSPs, was blocked by the slow intracellular calcium chelator ethyleneglycol-bis (β-aminoethyl)-*N, N, N′, N′*-tetra acetoxymethylester (EGTA-AM). The reliability was thus calcium-dependent. In the presence of EGTA-AM, transmission could fail after one or two bursts of spikes, suggesting that there is a limited releasable vesicle pool. The regularity was not blocked by the L-type calcium-channel antagonist nimodipine, suggesting that the calcium signal was related to that underlying transmitter release *(43).* This mechanism could thus act as a negative feedback, monitoring and replenishing the releasable transmitter pool in response to synaptic activity during different patterns of network outputs *(49).*

The reliability of other interneuron inputs have also been examined to some extent *(43).* SiIN and inhibitory ScIN inputs to motor neurons occurred consistently over repeated bursts, and they could thus contribute to sustained segmental network activity. However, EIN inputs to CCINs and excitatory CCIN and ScIN inputs to motor neurons depressed markedly over repeated bursts. These connections are thus unlikely to contribute to sustained segmental network activity.

With the exception of EIN inputs to motor neurons, the mechanisms underlying the activity-dependent plasticity have not yet been studied in detail. However, plasticity is not simply related to the stimulation frequency *(31,43,44),* and in contrast to most other systems *(46,50),* does not appear to be related to the initial release probability *(31,43,44).* Synaptic transmission may instead be actively regulated, possibly through effects on the transmitter-release machinery or calcium entry (*see,* for example *51,52*), and also through the activity-dependent effects on the maintenance of reliable transmission *(43,49).* This is not unexpected. The duration and frequency of network interneuron spiking must vary in order to generate motor outputs of different patterns and frequencies. Active mechanisms for regulating synaptic transmission are thus presumably required to allow synaptic transmission during dynamic network activity.

The Metaplasticity of Network Interneuron Synaptic Transmission

The expression of activity-dependent synaptic plasticity can be modulated *(10,11),* an effect that has recently been termed metaplasticity *(6,10).* The above analysis of activity-dependent synaptic plasticity was used as a basis for examining neuromodulator-evoked metaplasticity in the spinal cord. Two modulators were used, substance P, which increases the network burst frequency *(53),* and 5-HT, which slows it *(54).* The opposite network effects of these modulators allowed the potential role of metaplasticity in the regulation of the burst frequency to be examined (*see* Fig. 3).

Substance P and 5-HT both evoked physiologically relevant metaplastic facilitation of glutamatergic inputs from EINs to motor neurons *(44).* In both cases, effects occurred with stimulation frequencies of 10 Hz or greater. The effects differed, however, in that the substance P-mediated facilitation developed from potentiation of the initial EPSP in the train, whereas the 5-HT-mediated facilitation developed from a depression of the initial EPSP (*see* Fig. 3A). Substance P thus markedly facilitated the net glutamatergic input during spike bursts, whereas 5-HT "redis-

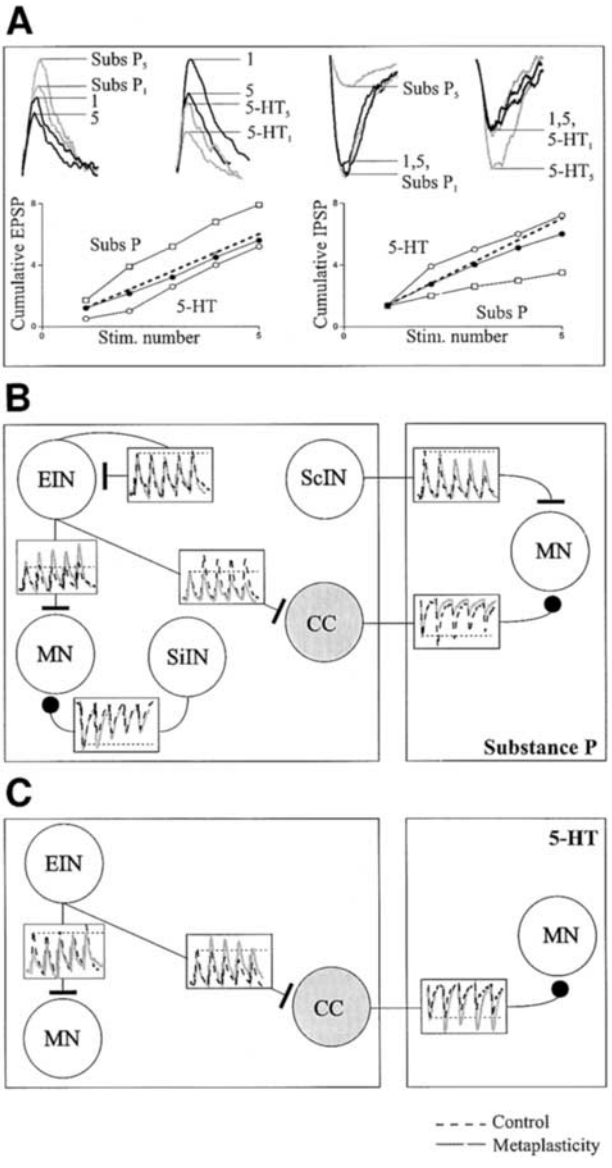

Fig. 3. The metaplasticity of network interneuron synaptic transmission. **(A)** Traces showing the first and fifth EIN-evoked EPSPs and CCIN-evoked IPSPs in a spike train in control (black lines), and the metaplastic effects of 5-HT or substance P (gray lines). The graphs show the cumulative EPSP and IPSP amplitudes in motor neurons in control (black symbols) and in the presence of substance P or 5-HT. The dashed line indicates the expected input if PSP amplitudes remained constant during the train. **(B)** Summary of the metaplastic effects of substance P on activity-dependent synaptic plasticity. The boxes show post-synaptic potentials evoked by a presynaptic spike train at 20 Hz in control (dashed lines) and in the presence of substance P (solid gray lines). **(C)** Metaplasticity evoked by 5-HT. Symbols and traces are as in (B). Traces on this figure do not reflect relative sizes, but are instead enlarged to the maximum size possible to facilitate the comparison of metaplastic effects. Horizontal dashed lines indicate the amplitude of the initial PSP in control.

tributed" it over the spike train *(55)*. 5-HT had the same effects on EIN inputs to CCINs. Substance P did not consistently affect this connection. Where an effect occurred it was a reduction of the initial EPSP followed by metaplastic depression (n = 2 of 6; Parker, unpublished observations). This is the opposite to the effect of substance P on EIN inputs to motor neurons, further emphasizing the role of neuron- and synapse-specific effects.

Substance P and 5-HT had opposite effects on glycinergic inputs from CCINs in contralateral motor neurons. Neither modulator significantly affected the initial IPSP amplitude. Substance P, however, evoked marked metaplastic depression during spike trains at frequencies of 10–20 Hz, whereas 5-HT evoked metaplastic facilitation at frequencies of 5–20 Hz (*see* Fig. 3A). The absence of an effect on the initial EPSP amplitude further supports the conclusion that activity-dependent plasticity is not simply related to changes in the initial release probability.

These metaplastic effects could contribute to the modulation of the network output *(53)*. Because the burst frequency is related to the level of excitatory drive *(33,56)*, the metaplastic facilitation of glutamatergic inputs by substance P is consistent with an increase in the network-burst frequency. This will depend, however, on the postsynaptic target in which the effect occurs, because potentiating inputs to crossing inhibitory neurons alone will potentiate contralateral inhibition and slow the burst frequency. The depression of CCIN inhibitory postsynaptic potentials (IPSPs) by substance P is also consistent with a reduction

of the network burst frequency, as reducing crossed inhibition will increase the burst frequency by allowing the contralateral network to become active sooner *(33)*.

The network effects of 5-HT were assumed to be explained solely by its effect on the slow calcium-dependent afterhyperpolarization (AHP_{KCa}; *57*). The marked synaptic effects of 5-HT, however, could also contribute. The potentiation of crossed inhibition by the facilitation of CCIN inputs could slow the burst frequency by delaying the activation of contralateral networks *(33)*. For glutamatergic inputs, the initial depression and subsequent facilitation of EIN-evoked EPSPs by 5-HT will influence the interpretation of synaptic inputs. The net excitatory drive will be reduced over short spike trains, but will approach control as the number of spikes in the train increases (*see* graph in Fig. 3A). The functional effect of this metaplasticity will thus be related to the number of spikes in the EIN burst.

Cooperative Interactions Between Metaplasticity and Cellular Modulation

Neuromodulators rarely have a single effect, but instead act on several distributed cellular and synaptic properties *(58)*. In the lamprey, this applies to both substance P and 5-HT, which, in addition to the synaptic effects described earlier, also modulate the excitability of spinal neurons *(57,59)*. As metaplasticity is frequency-dependent *(44)*, it could be influenced by the modulation of interneuron excitability, resulting in cooperative or antagonistic modulatory effects *(60)*.

Substance P increases EIN excitability *(59)*. The metaplasticity of inputs from EINs occurs at frequencies of 10 Hz or greater. Below this frequency, the increase in excitability will act independently to increase glutamatergic inputs. Above 10 Hz, however, metaplasticity and increased cellular excitability could act synergistically to potentiate EIN inputs. CCIN excitability is reduced by substance P *(59)*. This cellular effect could act synergistically with the metaplastic depression of CCIN inputs to reduce crossed inhibition. However, if the CCIN spike frequency falls below the level at which metaplastic depression occurs (<10 Hz), the reduced excitability will again act independently.

5-HT increases the excitability of motor neurons, LINs, and possibly CCINs by reducing the slow calcium-dependent afterhyperpolarization (AHP_{Kca}; *57*). Although assumed in EINs *(33)*, preliminary data suggest that 5-HT may not affect the AHP_{KCa} or excitability of these cells (Parker, unpublished observations). In the absence of an effect on EIN excitability, the 5-HT-mediated redistribution of glutamatergic inputs will occur independently. However, assuming that CCIN excitability is increased, it will act synergistically with the metaplastic facilitation of CCIN inputs to potentiate crossed inhibition.

Conclusions

Activity-dependent synaptic plasticity, neuromodulator-mediated metaplasticity, and cooperative cellular and synaptic interactions are thus potential mechanisms involved in the patterning and plasticity of network activity. The molecular and cellular mechanisms underlying these effects now need to be examined. As stated earlier, activity-dependent plasticity does not appear to be simply related to release probability or stimulation frequency, suggesting that synaptic properties are actively regulated. Metaplasticity could involve the activation of intracellular pathways that modulate transmitter-release mechanisms, releasable-vesicle pool sizes, or transmitter-replenishment mechanisms *(6,10,61)*.

Activity-dependent and metaplastic effects have only been examined in quiescent preparations. Their involvement during network activity thus needs to be determined. Computer simulations of the aforementioned data support the role of metaplasticity and cooperative interactions in regulating the ongoing burst frequency *(62)*. However, although supporting

the role of these effects, the modeling studies assume that the organization and properties of the physiological and simulated networks are similar, something that is currently uncertain (*see* above). In addition, they assume that cellular, activity-dependent, and metaplastic effects, which have largely been examined on interneuron inputs to motor neurons, can be extrapolated to connections made between segmental network interneurons. As shown in this and other reviews *(58),* this is an assumption that cannot be made with confidence. These effects must thus be examined directly. In particular, the experimental analysis of crossing inhibition has largely focused on inputs to and from CCINs. In light of the uncertain segmental role of the CCINs, conclusions based on these results can only be interpreted when their segmental or intersegmental role is established.

Neuropeptide-Mediated Modulation in the Spinal Cord

In addition to activity-dependent effects, plasticity can also be evoked by neuromodulatory transmitters that act through G proteins and second-messenger pathways to influence cellular and synaptic properties. Aminergic and metabotropic amino acid-mediated neuromodulation has been examined in detail in the spinal cord *(5).* The analysis of neuromodulation reviewed here has focused on the effects of neuropeptides. Although the location and molecular biology of spinal neuropeptides have been examined in detail *(63),* their physiological effects had largely focused on sensory inputs. The types, sequences, and locations of lamprey neuropeptides are similar to those in higher vertebrates *(64),* thus making the lamprey a relevant system in which to examine spinal peptidergic modulation.

Modulators can have distributed effects on cellular and synaptic properties *(58).* They can also act at different levels. In the spinal cord this could be on sensory inputs, descending inputs, or directly on the locomotor network. Peptidergic neuromodulation was examined by investigating the effects of six endogenous peptides (tachykinins, NPY, PYY, bombesin, CCK, and CGRP) on cellular and synaptic properties at these different spinal levels.

Sensory Modulation

The analysis of sensory modulation focused on inputs from cutaneous mechanosensory dorsal cells *(26).* These have large cell bodies in the midline of the spinal cord, and can thus be readily identified and recorded from. In addition, they make monosynaptic connections with giant spinobulbar interneurons *(26),* thus allowing sensory synaptic transmission to be examined.

Tachykinins are contained in a ventromedial spinal plexus, and in primary afferents and sensory interneurons in the spinal dorsal horn *(65).* The tachykinin substance P depolarized and increased the excitability of the dorsal cells, increased the action potential duration, and reduced the spike afterhyperpolarization (AHP; *66*). Certain of these effects were mediated by a pertussis toxin-insensitive G protein and the protein kinase C (PKC)-mediated inhibition of 4-aminopyridine-sensitive potassium conductances *(67).* Substance P also increased dorsal-cell excitability by reducing a post-tetanic afterhyperpolarization evoked by an electrogenic pump and a Ca^{2+}-activated potassium conductance *(68).* At the synaptic level, substance P reduced dorsal column-evoked inhibitory inputs to spinobulbar neurons, but potentiated excitatory dorsal column and dorsal root-evoked inputs. It did not, however, affect inputs from dorsal cells, suggesting that the potentiation of dorsal root and dorsal column-evoked EPSPs was due to an effect on nonmechanosensory (e.g., nociceptive or thermosensitive) inputs. This general potentiation of sensory cellular and synaptic properties was associated with the facilitation of skin stimulation-evoked reflex responses *(69).*

Bombesin, which is located in small diameter primary afferent axons with CGRP and 5-

HT *(70),* also increased dorsal-cell excitability and potentiated dorsal column-evoked inputs to spinobulbar interneurons *(69).* These excitatory effects were again associated with the facilitation of skin stimulation-evoked reflex responses *(69).* In contrast, CGRP did not directly affect sensory synaptic inputs, although it may indirectly facilitate these inputs by blocking endogenous tachykinin breakdown *(71).*

NPY and PYY are contained in interneurons in the dorsal column, and in descending reticulospinal neurons, respectively *(64,72).* Both peptides presynaptically reduced dorsal-root, dorsal-column, and monosynaptic dorsal-cell synaptic inputs to spinobulbar interneurons. NPY also reduced postsynaptic spinobulbar interneuron excitability *(72).* NPY co-localizes with gamma-amino butyric acid (GABA) in dorsal column interneurons *(72).* NPY and the $GABA_B$-receptor agonist baclofen additively inhibited sensory synaptic inputs, although the effect of NPY was smaller and longer-lasting. These inhibitory cellular and synaptic effects of NPY were associated with the reduction of skin stimulation-evoked reflex responses *(69).*

The Modulation of Descending Brainstem Inputs

Peptidergic effects were also examined on descending brainstem inputs to the spinal cord *(73).* With the exception of substance P, which evoked pre- and post-synaptic facilitation, the peptides all pre-synaptically reduced reticulospinal axon-evoked synaptic inputs (*see* Fig. 4). These effects were associated with the modulation of brainstem stimulation-evoked ventral-root responses *(73).* Neuropeptides thus functionally modulate descending inputs to the spinal cord.

Locomotor-Network Modulation

Locomotor-network modulation was examined by investigating peptide effects on NMDA-evoked ventral-root activity in the isolated spinal cord. Substance P increased the frequency and improved the regularity of network activity *(53).* This was associated with a range of cellular and synaptic effects in identified network neurons (*see* Table 2). CCK, NPY, and PYY did not directly affect network activity *(73).* CGRP may increase the network-burst frequency, again possibly through an indirect effect on endogenous tachykinin breakdown.

Conclusions

Endogenous neuropeptides thus affect cellular and synaptic properties at the sensory, descending brainstem, and locomotor-network levels. There is no common theme to the modulation. Substance P potentiates at all levels; NPY modulates descending and sensory inputs, but does not directly affect the locomotor network; whereas CGRP reduces descending inputs, but does not directly affect sensory or network properties. In addition to distributed effects at different spinal levels, the peptides also had distributed effects on cellular and synaptic properties at these levels. The selection of effects within and between levels could underlie flexibility in the evoked modulation. This could potentially occur through modulator interactions (*see* below). In addition, modulator release from separate modulator-containing systems may allow effects to be recruited spatially. Tachykinins, for example, could be released from primary afferents, sensory interneurons in the dorsal horn, or the ventromedial plexus *(65).* Finally, distributed effects could be concentration-dependent, possibly due to different receptor subtypes, thus allowing effects to be recruited as a function of the activity of modulator-containing neurons. Substance P illustrates the potential for concentration-dependent selection. Its mechanosensory effects increase in magnitude up to 100 nM, but are reduced or reversed at 1 μ*M (67,69).* In contrast, the magnitude and duration of its locomotor network modulation increases with increasing concentration *(53).* At nM levels, the mechanosensory and network modu-

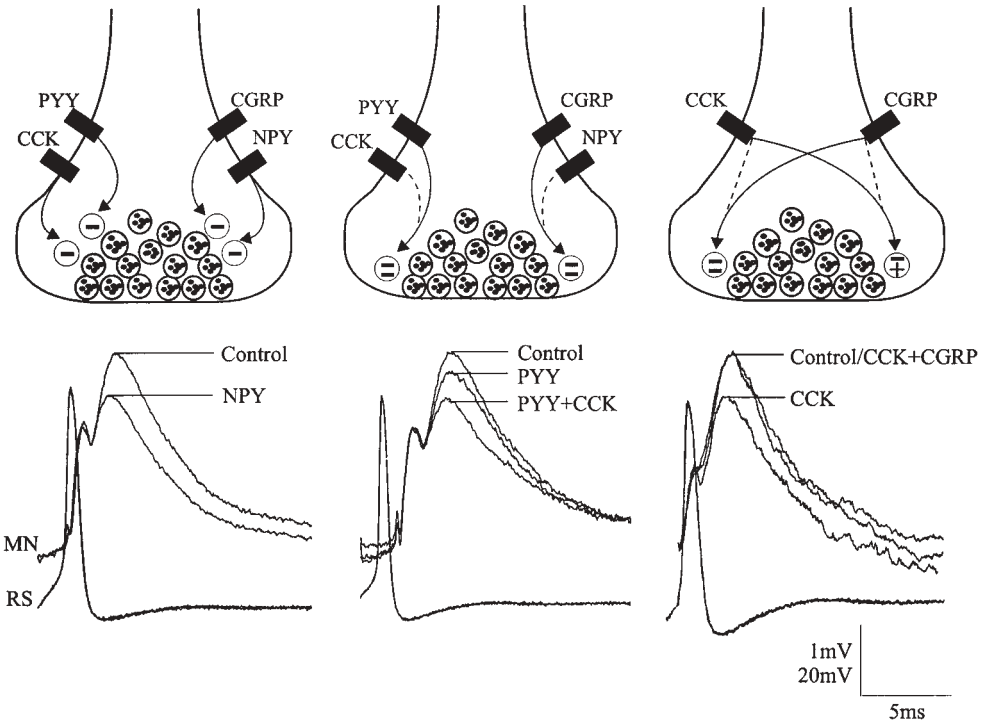

Fig. 4. Summary of the individual and interactive effects of neuropeptides on pre-synaptic reticulospinal inputs. – indicates an inhibitory effect on glutamatergic synaptic transmission, + a facilitating effect. Where interactive effects were examined, the solid line indicates the peptide that was applied first, the dashed line the peptide that was applied second. The top symbol in the circles represents the effect of the first modulator, the lower symbol that of the second modulator. The traces show examples of individual and interactive modulator effects examined by making paired recordings from reticulospinal axons and motor neurons.

lation will thus occur together, whereas at μ*M* levels mechanosensory effects will be reduced, resulting in a dominance of the network effects.

Long-Term Effects of Substance P on the Locomotor Network

The direct effects of substance P on the locomotor network were surprising *(53).* This aspect has thus dominated the analysis of neuropeptide-mediated spinal plasticity and will be described in detail.

Bath application of substance P for 10 min resulted in a concentration-dependent increase in the frequency of NMDA-evoked ventral-root bursts (*53; see* Fig. 5). In addition to the magnitude of this effect, its time-course was also concentration-dependent. With nM concentrations of substance P (10–100 nM) recovery occurred after washing for 1–5 h. However, with 1 μ*M* substance P, a physiological concentration for a neuropeptide *(74),* no appreciable recovery occurred even washing in excess of 24 h. The burst-frequency modulation was blocked by the tachykinin receptor-antagonist spantide II.

In addition to increasing the burst frequency, substance P also made the activity more regular. This was shown quantitatively by a concentration-dependent reduction of the coefficient of variation (CV; standard deviation

Table 2
Summary of the Distributed Cellular and Synaptic Effects of Substance P on Motor Neurons and Identified Presumed Locomotor-Network Interneurons, and on Sensory and Descending Reticulospinal Inputs[a]

Property	Effect	Cell or synapse type	Duration
Membrane potential	Depolarisation and oscillations	MN, LIN CCIN, EIN	10–20 min
Input resistance	Not affected	MN, LIN, CCIN, EIN	–
Excitability	Increased	MN, LIN EIN	~ 1 h
Excitability	Decreased	CCIN	~ 1 h
Slow AHP (Kca)	Reduced	MN, LIN	~ 1 h
Slow AHP (Kca)	Not affected	CCIN, EIN	–
Low-frequency glutamatergic synaptic transmission	Potentiated	EIN-MN, RS-MN	~ 1–2 h
Low-frequency glutamatergic synaptic transmission	Depressed	ScIN-MN, EIN-CCIN?, DC to contralateral GI	~ 1 h
Low-frequency glutamatergic synaptic transmission	Not affected	EIN-CCIN?, DC to ipsilateral GI	–
Glutamatergic metaplasticity	Facilitation	EIN-MN, ScIN-MN?	?
Glutamatergic metaplasticity	Not affected (or depression?)	EIN-CCIN	–
Postsynaptic NMDA responses	Potentiated	MN, CCIN	~ 1–2 h
Low-frequency glycinergic synaptic transmission	Not affected	LIN-CCIN, CCIN-MN, SiIN-MN	–
Low-frequency glycinergic synaptic transmission	Potentiated	SiIN-MN	~ 1 h
Low-frequency glycinergic synaptic transmission	Depressed	ScIN-MN	?
Glycinergic metaplasticity	Depressed	CCIN-MN	?
Glycinergic metaplasticity	Facilitated?	SiIN-MN	?
Postsynaptc $GABA_A$ and $_B$ responses	Not affected	MN	–

[a] The abbreviations are defined in the legend to Fig. 1. Where effects are variable, they are repeated under different sections.

of the cycle duration/mean cycle duration). Substance P thus made the interval between successive bursts more constant (*see* Fig. 5B). This effect was also long-lasting with 1 μ*M* substance P, and was blocked by spantide II.

Faster network activity was not necessarily more regular. This was shown by the lack of a correlation between the burst frequency and CV *(53).* The burst regularity effect is thus not simply an epiphenomenon associated with the burst frequency modulation, but a separate specific effect of substance P on the locomotor network. This conclusion was supported by differences in the mechanisms underlying the two effects (*see* below).

Cellular and Synaptic Mechanisms Underlying the Network Modulation

Substance P thus has two long-term effects on the locomotor network, an increase in the burst frequency and an improvement in the burst regularity. After characterizing the network modulation, the next step was to examine its underlying cellular and synaptic basis. Because the long-term effect was of particular

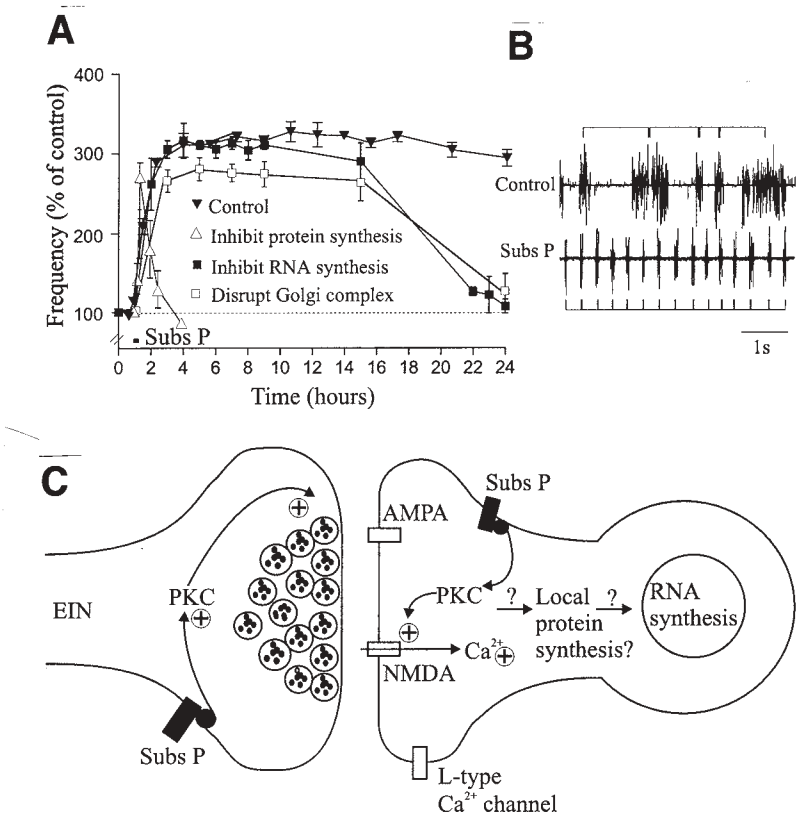

Fig. 5. Summary of the long-term effect of substance P on the locomotor-network burst frequency. **(A)** Graph showing the effects of substance P (1 μ*M*, applied for 10 min) on the frequency of NMDA-evoked ventral-root bursts in control, when protein synthesis was blocked by translation or transcription inhibitors, or when the Golgi complex was disrupted by Brefeldin A. **(B)** Traces showing control activity and activity 9 h after substance P wash-off. off. The bars above and below the traces represent the cycle duration (defined as the midpoint between successive bursts in a single ventral root). Notice that in addition to increasing the burst frequency, substance P also makes the cycle duration more constant, thus improving the burst regularity. **(C)** Diagram representing a glutamatergic EIN terminal synapsing onto a post-synaptic motor neuron. Pre-synaptically, substance P acts through protein kinase C (PKC) to potentiate glutamate release. Post-synaptically, it acts through PKC to potentiate NMDA-mediated responses. This is presumed to evoke the increased Ca^{2+} levels underlying the burst-frequency modulation. The potentiation of NMDA responses underlies the induction, but not maintenance, of the burst-frequency modulation. The maintenance requires two phases of protein synthesis, the first of which may occur locally in dendrites. + indicates a potentiating effect.

interest, experiments focused on the effects of 1 μ*M* substance P.

Insight into a cellular mechanism underlying the burst frequency modulation came from examining network activity elicited by the non-NMDA receptor-agonist kainate *(53)*. As with NMDA-evoked activity, substance P increased the frequency and improved the regularity of kainate-evoked activity. The burst-frequency modulation, however, failed to develop when NMDA receptors were blocked by the antagonist AP5, although the burst-regularity modulation persisted. The burst-frequency modulation was thus NMDA-dependent, the burst-regularity modulation NMDA-independent, supporting the

conclusion that these are two separate effects of substance P on the locomotor network.

The NMDA-dependence of the burst-frequency modulation was associated with the potentiation of cellular responses to NMDA, but not α amino-3-hydroxy-5-methyl-4-isoxazole-propionic acid (AMPA), in network neurons *(53).* The potentiation of NMDA responses was PKC-dependent, but was not associated with the modulation of the glycine site or the Mg^{2+} block of the receptor *(53).* The modulation of cellular responses to NMDA could account for the NMDA-dependence of the burst-frequency modulation. However, potentiated NMDA responses recovered to control after washing for 30 min-2 h, and the long-term network modulation was not reversed when NMDA receptors were blocked 3 h after substance P application. These results thus suggest that tonic NMDA-receptor modulation is not required to maintain the burst-frequency modulation.

The potentiation of NMDA responses was associated with the facilitation of monosynaptic EPSP amplitudes from glutamatergic reticulospinal axons and EINs *(59).* The analysis of spontaneous miniature EPSPs suggested that substance P also presynaptically potentiated glutamate release, an effect that was largely independent of calcium entry *(59).* This increase in glutamatergic inputs could account for the burst-frequency modulation *(33).* Because the burst-frequency modulation was NMDA-dependent, the post-synaptic potentiation of NMDA responses was likely to be of particular importance. A link between the NMDA-receptor and burst-frequency modulation was suggested by two sets of experiments. Firstly, although the PKC antagonist chelerythrine did not consistently affect ongoing network activity, it blocked the NMDA-receptor potentiation and the burst-frequency modulation. Neither effect was shared by protein kinase A or G (PKA/PKG) inhibitors *(53).* The burst-frequency and NMDA-receptor modulation thus have the same intracellular pathway, providing correlational support for a link between these effects. The second approach took advantage of the fact that NMDA receptors are permeable to Ca^{2+}. The potentiation of NMDA responses should thus increase Ca^{2+} levels in network neurons, an effect that can contribute to network plasticity *(75).* The role of Ca^{2+} in the burst-frequency modulation was examined using the slow intracellular calcium-chelator EGTA-AM. Although EGTA-AM did not significantly affect monosynaptic EPSP amplitudes or ongoing network activity *(53),* presumably because it cannot buffer the rapid Ca^{2+} transients required to evoke transmitter release *(76),* it blocked the induction of the burst-frequency modulation *(53).* The burst-frequency modulation was thus dependent on increased intracellular Ca^{2+} levels. Although other sources of Ca^{2+} could contribute (*75;* but not L-type Ca^{2+} channels, *see* below), this result again suggests that the NMDA-receptor modulation and burst-frequency modulation are related.

The PKC-mediated potentiation of NMDA receptors and the resulting increase in intracellular Ca^{2+} levels is suggested to account for the burst-frequency modulation (*see* Fig. 5). However, because the presynaptic and NMDA-dependent postsynaptic modulation of glutamatergic inputs recovered within 2 h of substance P application, this scheme can only account for the induction, not the maintenance, of the burst-frequency modulation. Substance P has a number of other cellular and synaptic effects (*see* Table 2). These are consistent with the network modulation, at least in the context of currently assumed network properties (*see* above). The potentiation of glutamatergic inputs is consistent with an increase in burst frequency (*see 33*). Although inhibitory synaptic transmission was initially not affected *(59),* this analysis focused on LIN and CCIN inputs, which may not be of relevance to the segmental network (*see* above). The subsequent analysis of the proposed segmental homologs of these cells, the SiINs and ScINs, suggests that inhibitory inputs are potentiated and depressed, respectively (Parker, unpublished observations). The SiIN effect could potentiate feedforward ipsilateral inhibition to terminate ipsilateral activity earlier, whereas the ScIN

effect could reduce crossed inhibition to allow contralateral networks to become active sooner. Both effects are consistent with an increase in the burst frequency. These results again emphasize the importance of identifying the relevant interneurons in the segmental network, and in identifying individual cells and synapses when examining network activity. However, the effects shown in Table 2 either recovered within 2 h of substance P application, and thus cannot maintain the burst-frequency modulation, or recordings were not maintained for sufficient time to allow the extent of recovery to be examined. These aspects thus require further examination.

Maintenance Mechanisms Underlying the Burst-Frequency Modulation

Although cellular mechanisms are currently lacking, insight into the maintenance of the burst-frequency modulation was obtained at the network level. Two aspects associated with long-term changes in other systems were examined. Firstly, the role of tonic protein kinase activation *(77,78)* was investigated by applying protein kinase inhibitors 3–4 h after substance P, i.e., when the proposed maintenance phase had begun. PKA, PKG, PKC, or CAM kinase inhibitors did not affect the burst-frequency modulation at this time. There is thus no evidence as yet to support a role for tonic protein kinase activation in the maintenance of the burst-frequency modulation.

The second approach examined the role of protein synthesis. This maintains long-term plasticity in several systems *(3,79).* The maintenance of the burst frequency modulation required two phases of protein synthesis *(80).* The first of these (the intermediate phase), developed 2–3 h after substance P application. This phase was consistently blocked by translation inhibitors, which prevent protein synthesis form mRNA, but not by transcription inhibitors, which block RNA synthesis and thus subsequently affect protein synthesis. Transcription inhibitors, however, usually reversed the burst-frequency modulation 15–20 h after substance P application, thus suggesting that there is a late phase of protein synthesis. Because the intermediate phase was not affected by transcriptional inhibitors, it apparently does not require *de novo* RNA synthesis. Similar *de novo* RNA synthesis-independent phases also occur in long-term facilitation in *Aplysia (81),* and hippocampal long-term potentiation *(82).* It may thus be a common intermediary stage in long-term plasticity.

Protein synthesis from pre-existing mRNA is supported by the presence of mRNA and its translation machinery in the dendrites of cortical, cerebellar, and hippocampal neurons *(83).* mRNAs for several proteins have been identified, the transport and translation of which can be triggered by synaptic activation *(83).* Interestingly, mRNA for the α subunit of the glycine receptor is present in the dendrites of rat spinal ventral-horn neurons *(84),* suggesting that the local-transmitter receptor synthesis could occur in the spinal cord. Local-protein synthesis could stabilize plasticity while genomic activation and protein transport occur. Alternatively, it may "tag" activated synapses to allow their specific regulation *(85).*

Support for local-protein synthesis in the intermediate maintenance phase of the burst-frequency modulation was obtained using the plant toxin Brefeldin A. This disrupts the Golgi apparatus, and thus affects the processing and targeting of proteins to cellular locations *(86).* Brefeldin A mimicked the effects of transcriptional inhibitors, blocking the late but not the intermediate maintenance phase (*80; see* Fig. 5). The intermediate phase thus did not require a functional Golgi complex, suggesting that proteins were synthesized in regions of the cell in which they were required. mRNA is assumed to be targeted specifically to dendrites *(83),* and thus dendritic synthesis is a possibility. mRNA is also present in axons in the hypothalamo-hypophyseal tract and the olfactory bulb, although the absence of ribosomes make its functional relevance uncertain *(83).* However, discrete ribosomal plaques are found in goldfish Mauthner axons and in rabbit and rat spinal axons *(87),* and protein synthesis occurs

in invertebrate axons *(88,89).* Local axonal protein synthesis thus cannot be ruled out.

The Role of Calcium in the Maintenance of the Burst-Frequency Modulation

Increased intracellular Ca^{2+} levels are required for the burst-frequency modulation (*see* Fig. 5). Ca^{2+} influx through NMDA or L-type calcium channels can activate specific intracellular pathways and transcription factors to influence gene expression *(75,90).* For example, NMDA receptors and L channel-mediated Ca^{2+} entry activates serum response element (SRE)-mediated transcription in hippocampal neurons, whereas only Ca^{2+} entry through L channels potently stimulates Ca^{2+} or cAMP response element (CRE)-mediated transcription *(90).* The CRE is activated by the cAMP-response element binding protein (CREB), and the CREB-binding protein (CBP) may underlie Ca^{2+} entry-dependent differences in gene expression *(91).*

Although the burst-frequency modulation is NMDA-dependent (*see* Fig. 5), it was not affected by the L-type Ca^{2+} channel antagonist nimodipine. L-type channels are thus presumably not involved (Parker, unpublished observations). Although Ca^{2+} release from intracellular stores cannot be ruled out, NMDA receptors thus appear a likely source for the increased intracellular Ca^{2+}. MAP kinase and SRE-mediated transcription are thus potential targets in the molecular analyses of the long-term burst-frequency modulation *(75).*

Modulation of the Burst Regularity

Although relatively little information is available on the burst-regularity modulation, several of its properties have been identified at the network level *(53).* Firstly, while the induction of the burst-regulatory modulation was NMDA-independent, it required that the network was active when substance P was applied *(53).* Neither effect was shared by the burst-frequency modulation, again emphasizing that separate mechanisms underlie these two network effects. Secondly, the burst-regularity modulation was blocked by PKA antagonists. It thus has a different intracellular pathway to the burst-frequency modulation. Finally, protein-synthesis inhibitors did not affect the maintenance of the burst-regularity modulation. PKA inhibitors, however, which do not significantly affect control network activity, reversed the burst-regularity modulation when applied up to 8 h after substance P *(80).* There may thus be a requirement for tonic PKA activation. Tonic cAMP activation underlies long-term facilitation in *Aplysia (77)* and hippocampal LTP *(92),* although in both cases protein synthesis is required.

Conclusions

The adult locomotor network is thus subject to long-lasting neuropeptide-mediated modulation. This may be related behaviorally to migration, when adult lampreys swim long distances to upstream spawning grounds *(93).* This possibility is supported by the absence of the network modulation in transforming lampreys (i.e., those developing from the larval to adult state), which do not undergo upstream migrations (Parker, unpublished observations).

Cellular mechanisms underlying the burst-regularity modulation and the maintenance of the burst-frequency modulation still require identification. This analysis should focus on the smaller segmental network interneurons (SiINs and ScINs), which may be more appropriate targets for segmental modulation than the LINs or CCINs, the interneurons that were examined initially *(29).* The role of protein synthesis in the maintenance of the network modulation must also be verified. The evidence for this currently relies on the use of transcription and translation inhibitors. These have toxic or nonspecific effects. Although obvious toxic effects were not seen in experiments using these inhibitors, conclusions based solely on their use can only be considered as preliminary.

Modulator Interactions and the Regulation of Spinal Plasticity

The nervous system contains a large number of putative neuromodulators. These are often co-localized, providing the opportunity for them to interact. Even without co- or simultaneous release, the "modulatory tone" resulting from their slow effects could allow interactions between modulators whose release is temporally or spatially independent. Modulator interactions appear to be common *(8,9).* Although their functional relevance is not well-understood, the potential complexity is daunting. Depending on previous or subsequent inputs, these interactions can make modulation either plastic or modulatory. The functional effects of these interactions and their underlying mechanisms are thus relevant components to our knowledge of nervous-system plasticity. Two examples of modulator interactions have been examined in the spinal cord.

Interactive Peptidergic Modulation of Descending Brainstem Inputs

The interactive effects of four neuropeptides were examined on descending reticulospinal inputs: CCK and PYY, which are contained in descending reticulospinal axons ("brainstem peptides), and CGRP and NPY, which are contained in primary afferents and sensory interneurons, respectively ("sensory peptides"; *see 73* for references). Each peptide individually reduced the amplitude of reticulospinal-evoked synaptic inputs *(73).* In interaction, the brainstem peptides CCK and PYY had additive inhibitory effects, as did the sensory peptides NPY and CGRP. The brainstem peptides also had additive inhibitory effects when applied in the presence of the sensory peptides. However, in the presence of the brainstem peptides, the sensory peptides could evoke a novel potentiating effect that reversed the initial depression (*see* Fig. 4). Sensory neuropeptides, possibly released as a result of strong sensory stimulation *(7),* can thus override the depression of reticulospinal inputs, to potentiate descending inputs to the spinal cord *(73).*

The peptidergic modulation of reticulospinal inputs is thus dependent on the previous modulation of the connection. All single and interactive peptide effects appear to be mediated presynaptically, but independently of an effect on the reticulospinal action potential. Independent and interactive effects may thus converge on presynaptic terminals to influence transmitter release, possibly through a modulatory effect on the transmitter-release machinery *(51,52).*

Aminergic and Peptidergic Interactions in the Gating and Breaking of Long-Term Network Plasticity

The second example of modulator interactions examined the effects of the amines 5-HT and dopamine on the network effects of substance P (Svensson and Parker, unpublished observations; *see* Fig. 6). These three modulators are contained in a ventromedial spinal plexus, although their exact distribution in the plexus is unknown. 5-HT and dopamine appear to co-localize in plexus neurons *(94),* whereas tachykinins co-localize to a limited extent with 5-HT. The data suggest that there may be three populations of neurons, one containing 5-HT, dopamine, and tachykinins, one 5-HT and dopamine, and one just tachykinins. The location of these modulators in the plexus provides the opportunity for them to interact, either through their co-localization and release, or, if stored and released independently, through their paracrinic release and relatively slow effects.

Pre-application of 5-HT blocked the induction of the substance P-mediated burst-frequency modulation. 5-HT did not reverse the burst-frequency modulation when it was applied 2 h after substance P (i.e., when the intermediate protein synthesis-dependent phase had begun), suggesting that it cannot reverse the burst-frequency modulation. The inhibition of the induction of burst-frequency

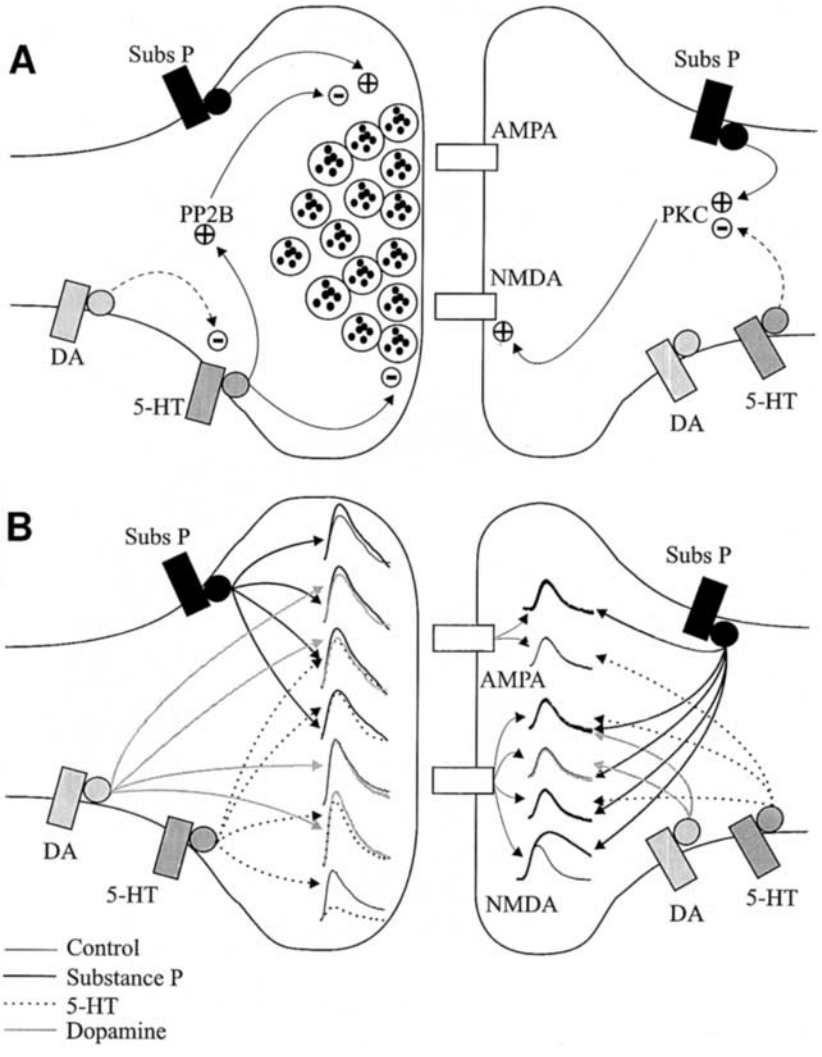

Fig. 6. **(A)**Summary diagram of the interactive effects of 5-HT and dopamine (DA) on substance P-evoked modulation of glutamatergic inputs from EINs. Pre-synaptically, 5-HT may act through protein phosphatase 2B (PP2B) to block the substance P-mediated potentiation of glutamate release. It also directly pre-synaptically inhibited EIN-evoked synaptic transmission. Post-synaptically, 5-HT acted through an unknown mechanism to block the substance P and phorbol ester-induced PKC-mediated potentiation of NMDA responses. 5-HT did not, however, directly effect NMDA responses. DA did not directly affect EIN-evoked synaptic transmission pre-synaptically or the effects of substance P, but acted through an unknown mechanism to inhibit the direct pre-synaptic inhibitory effect of 5-HT, and the interactive inhibition of the substance P-mediated potentiation. DA did not, however, affect post-synaptic responses to NMDA or the 5-HT-mediated inhibition of their potentiation by substance P. – indicates an inhibitory effect, + a facilitating effect. **(B)** Diagram showing the individual and interactive effects of substance P, 5-HT and dopamine on pre- and post-synaptic glutamatergic synaptic transmission.

modulation was mediated through an inhibitory effect of 5-HT on the substance P-mediated potentiation of NMDA responses required to induce the burst-frequency modulation (*see* Fig. 5). 5-HT also blocked the phorbol ester-induced potentiation of NMDA responses, suggesting that it inhibited the substance P-mediated modulation through an intracellular interaction on PKC-mediated pathways (*see* Fig. 6). Although the mechanisms underlying this intracellular interaction are unknown, it is not PKA, PKG, or protein phosphatase-dependent. However, 5-HT also blocked the substance P-mediated presynaptic facilitation of EIN-evoked EPSPs. This effect appeared to be mediated through a protein phosphatase 2B-mediated mechanism that presumably inhibits the presynaptic PKC-mediated effects of substance P (*see* Fig. 6). 5-HT thus brakes the tachykinin-mediated cellular, synaptic, and network modulation.

Distributed modulatory effects have been suggested to allow flexibility in evoked plasticity *(5,58),* possibly by selecting components from an individual modulators repertoire. Direct support for this was obtained by investigating the interactive effects of 5-HT and dopamine on the substance P-mediated modulation. Dopamine and 5-HT together occluded any significant effect of substance P on postsynaptic NMDA responses or the network burst-frequency modulation. Dopamine, however, blocked the presynaptic inhibitory effect of 5-HT, to gate the short-term presynaptic facilitation by substance P. Because the postsynaptic inhibitory effect of 5-HT on the NMDA receptor potentiation was not relieved, however, this short-term synaptic effect occurred in the absence of the long-term network plasticity.

As 5-HT and dopamine co-localize in plexus neurons, mechanisms must exist for their differential release if the gating and braking effects shown here are to occur physiologically. These mechanisms could include the storage and release of 5-HT and dopamine from separate vesicle populations *(7),* or the modulation of the contents or release of dense-cored vesicles *(95,96).* Finally, modulator release from other sources than the ventromedial plexus could contribute to these interactive effects. Because exogenous modulator application does not necessarily mimic endogenous effects *(97),* the source, release, and endogenous effects of these modulators now requires examination.

Conclusions

Modulator interactions thus evoke dynamic cellular, synaptic, and network plasticity. The potential for these interactions suggests that examining individual modulator effects in isolation may provide only partial insight into their functional roles. The interactive presynaptic modulation of reticulospinal inputs suggests that interactions can occur through convergent effects on a single cellular property. Conversely, the interactive effects of 5-HT and dopamine on the substance P-mediated modulation suggest that dynamic network plasticity results from the selection of distributed effects of a single modulator. Several mechanisms could account for these interactions *(9),* including effects on endogenous modulator release, the modulation of uptake or breakdown mechanisms, the affinity of modulators for their receptors, or, as suggested here, by interactions between intracellular pathways.

General Conclusions

The following general aspects are illustrated in this review.

1. Physiologically relevant activity-dependent synaptic plasticity occurs at connections between presumed interneurons in the locomotor network. This supports the suggested role of activity-dependent plasticity (and metaplasticity) in the patterning and plasticity of network outputs *(40).*
2. In analogy to metaplasticity, neuromodulation can be modulated. These effects occur in several systems *(8,9),* and could thus be common occurrences in the nervous system. Modulator interactions can converge on individual cellular or synaptic properties, or can select from the dis-

tributed effects of a single modulator. Previous or subsequent modulatory inputs can thus determine net functional effects.

3. Neuromodulator and activity-dependent effects diverge to several cellular and synaptic properties at different spinal levels (network, sensory, or descending). The selection of cellular and synaptic components within and between these levels may allow flexibility in the evoked plasticity.
4. The mechanisms underlying the induction of the burst-frequency modulation are qualitatively similar to those underlying long-term changes thought to underlie learning and memory in invertebrate and vertebrate systems *(3,79).* These mechanisms may thus be conserved not only across species, but also across functions, inducing long-term plasticity irrespective of the trigger, function, or region in which it is evoked *(98,99).*
5. As predicted from invertebrate *(58)* and vertebrate systems *(46),* individual cellular and synaptic properties and their plasticity are unique. Extrapolations from unidentified neurons and extracellularly evoked synaptic effects thus provide limited insight into the cellular and synaptic mechanisms underlying network activity and its plasticity.

Although simplicity is elegant, it can also be misleading. Linear chains of cause and effect and extrapolated cellular and synaptic properties are assumed to reduce the "explanatory gap" *(100)* between molecular, cellular, or synaptic and network or behavioral effects. Attempts to provide detailed information on network components and their properties have conversely been referred to derisively as "philatelism" (*see* comments to *38*). Simplifications are a necessary first step. However, even when examined *in situ,* isolated molecular, cellular, or synaptic properties cannot be assumed to reflect physiological roles in integrated networks, or to "explain" the patterning or plasticity of a network output involving different components at several levels. In addition, single-network components exhibit different forms of plasticity that could interact in vivo. For example, the plasticity of EIN inputs to motor neurons could involve the modulation of pre- and post-synaptic excitability, tonic neuromodulator-mediated synaptic modulation, activity-dependent synaptic plasticity, and neuromodulator-mediated metaplastic and cooperative interactions. In addition to the diverse plasticity of single-network components, mechanisms that evoke plasticity can also diverge to affect several cellular and synaptic properties at different levels. Although a single property or effect could contribute to the patterning or plasticity of the network output under certain conditions, the independent or interactive effects of the entire ensemble should be evaluated if the goal is to obtain genuine insight into the molecular, cellular, and synaptic basis of network activity.

An analysis of all properties and their plasticity may, however, be impossible. Computer simulations allow the evaluation and prediction of effects, although caution again has to be exercised in the level of explanation assumed *(101).* The effects of 5-HT in the lamprey provide an example of how seductive extrapolated simulations can be. The assumed reduction of the AHP_{Kca} in all network neurons, which may not occur physiologically (Parker, unpublished observations), mimics the network effects of 5-HT, even though its tonic and metaplastic synaptic effects were not included. This required simplifying assumptions in the network simulations, however, notably the removal of ipsilateral inhibition *(33*).

Functional Relevance of Spinal Plasticity

Spinal networks are thus subject to a variety of activity-dependent, neuromodulator, and interactive plasticity. Short-term individual or interactive plasticity may evoke transient adaptations of sensory inputs, descending inputs, or the locomotor network in response to changes in internal or external environments. Long-term effects may underlie developmental or seasonal changes *(5).* Plasticity may also be homeostatic *(102),* or homeodynamic *(25),* and actively adapt networks to alterations in their inputs. This may contribute to the functional reorganization of circuitry and functional recovery after spinal *(103)* or

cortical injury *(104,105)*. The underlying mechanisms are not known in detail, but training and pharmacological-induced effects could be related to activity-dependent or metaplastic effects *(104,105)*.

The lamprey regains locomotor function after complete spinal lesions *(106,107)*. It thus provides a basis for examining the role of adaptive mechanisms in functional recovery. Although the recovery is associated with axonal regeneration across lesion sites, this regeneration alone cannot account for the recovery *(106,107)*. Other factors that could contribute include the plasticity of descending propriospinal systems, the plasticity of sensory or regenerated descending inputs *(106)*, or changes in network properties caudal to lesion sites *(103)*. That caudal changes may contribute is supported by preliminary data that suggests that following chronic block of synaptic transmission with tetropotoxin (TTX), there is an upregulation in the amplitude and possible frequency of spontaneous miniature EPSPs and IPSPs (Parker, unpublished observations). Insight into this and other potential mechanisms may allow endogenous effects to be utilized in promoting functional recovery. By harnessing the properties of endogenous spinal circuitry, the burden on other approaches could be eased, for example by reducing the extent of axonal regeneration required or the complexity of prosthetic devices *(103)*. For this to be possible, however, detailed information is required on adaptive plasticity mechanisms, as well as on the mechanisms that activate and pattern basic spinal outputs.

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