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Neuropeptide Modulation of Microcircuits

Michael P. Nusbaum[1] and **Dawn M. Blitz**[2]

Michael P. Nusbaum: nusbaum@mail.med.upenn.edu

[1]Department of Neuroscience, Perelman School of Medicine, University of Pennsylvania, 215 Stemmler Hall, Philadelphia PA, 19104-6074, Phone: 215-898-1585, FAX: 215-573-9050

[2]Department of Zoology, Miami University, Oxford, OH 45056

Abstract

Neuropeptides provide functional flexibility to microcircuits, their inputs and effectors by modulating pre- and postsynaptic properties and intrinsic currents. Recent studies have relied less on applied neuropeptide and more on their neural release. In rhythmically active microcircuits (central pattern generators, CPGs), recent studies show that neuropeptide modulation can activate particular activity patterns by organizing specific circuit motifs. Neuropeptides can also modify microcircuit output indirectly, by modulating circuit inputs. Recently elucidated consequences of neuropeptide modulation include changes in motor patterns and behavior, stabilization of rhythmic motor patterns and changes in CPG sensitivity to sensory input. One aspect of neuropeptide modulation that remains enigmatic is the presence of multiple peptide family members in the same nervous system and even the same neurons.

Introduction

Neuropeptides are pervasive intercellular signaling molecules that function as neurotransmitters, via paracrine actions, and/or as circulating hormones. With few exceptions [1,2], neuropeptides act via G protein-coupled receptors (GPCRs) and thereby cause relatively long-lasting changes in intrinsic and/or synaptic properties [3,4]. There is a growing literature documenting the ability of neuropeptides to modulate microcircuit output, either directly [4–11, 12••,13••,16•,18••,19••,20••,22••, 23••, 24] or by influencing inputs to these circuits [25–27,28••,29] (Fig. 1a). Neuropeptides thus contribute substantially to the multifunctional nature of microcircuits, enabling the generation of distinct output patterns when influenced by different neuromodulators [10,11,30–32]. Here we focus on recent work providing new insights into neuropeptide modulation of microcircuit output, particularly with respect to sensory (olfaction, proprioception) and rhythmic motor systems.

Presynaptic peptidergic actions

Many neuropeptide actions are shared with those mediated by metabotropic receptors for small molecule transmitters. For example, they all commonly act via GPCRs, enabling changes in the cellular and synaptic properties of target neurons. One shared site of action is the presynaptic terminal, where metabotropic actions are well-established to regulate

Correspondence to: Michael P. Nusbaum, nusbaum@mail.med.upenn.edu.

neurotransmitter release [33] (Fig. 1b,c). It is important to note that transmitter release can also be regulated by postsynaptic activation of retrograde messengers, some of which are neuropeptides [34,35•] (Fig. 1b).

With respect to microcircuit operation, previous studies have primarily focused on presynaptic modulation of transmitter release by small molecule transmitters [29,36–38, 39••,40], with fewer studies establishing a comparable action by neuropeptides [5]. However, several recent papers document the ability of neuropeptides to presynaptically modulate neurotransmitter release in the microcircuit context [29,35•,41, 42•,43], particularly in the olfactory system.

Peptidergic presynaptic modulation plays multiple roles in the first-stage microcircuit in the olfactory system of both invertebrates (antennal lobe) and vertebrates (olfactory bulb) [18••, 23••,29,44••,45,46,47••]. There is both up- and downregulation of synaptic transmission from olfactory receptor neurons (ORNs) onto 2nd order neurons as well as at later stages of processing within these olfactory microcircuits. These recent examples of peptidergic presynaptic modulation influence olfactory cue recognition and olfactory guided behaviors such as feeding, and identifying known conspecifics [18••,23••,29,44••,45,46,47••]. Yet more complexity will undoubtedly be identified in these circuits, as presaged by the recent finding that the fly antennal lobe contains additional peptides [48].

Modulation of neuropeptide release

Presynaptic modulation can in turn regulate peptidergic transmission (Fig. 1c). For example, presynaptic inhibition of neuropeptide release in a *C. elegans* ORN is pivotal to food searching and odor adaptation behaviors [18••]. This regulation, mediated by a (peptidergic) feedback synapse, limits the duration of ORN peptide release and thereby limits the local search for food and promotes odor adaptation. Whether this feedback inhibition also inhibits cotransmitter (glutamate) release was not determined, although one indirect measure of transmitter release (amplitude and duration of intracellular Ca^{2+} transients) suggests that glutamate release is also compromised.

Presynaptic modulation can also selectively inhibit peptidergic cotransmission (Fig. 1c). In the isolated crab stomatogastric nervous system (STNS), an identified muscle stretch-sensitive sensory neuron (GPR neuron) inhibits the axon terminals of a multi-transmitter projection neuron (MCN1), weakening MCN1 peptidergic modulation without altering the strength of its GABAergic transmission [39••]. MCN1 activity drives the gastric mill (chewing) rhythm, in vitro and in vivo [49,50]. The selective regulation of peptidergic cotransmission enables the sensory input to have a phase-specific influence on the gastric mill rhythm [39••]. The intracellular mechanism underlying this event is not known, but likely involves an aspect of the neuropeptide release process that is not shared with that for small molecule transmitter release [51–53].

Interestingly, Shakiryanova et al. [54•] recently established that synaptic neuropeptide release can be evoked from a *Drosophila* motor neuron in the absence of extracellular Ca^{2+} by octopamine (the arthropod equivalent of norepinephrine) application. Octopamine evokes this release via a parallel activation of cAMP-activated protein kinase and Ca^{2+} release from intracellular stores. Whether octopamine also enables extracellular Ca^{2+}-independent release of the small molecule cotransmitter (glutamate) was not determined.

Modulating microcircuit organization and dynamics

Several novel insights into the circuit consequences of peptidergic modulation were recently elucidated in two well-defined CPG systems that generate rhythmic motor patterns

underlying feeding-related movements in the mollusc *Aplysia californica* [55] and the STNS of crabs and lobsters [10,14]. The studies highlighted below focus on the acute actions of peptidergic modulation, but it is noteworthy that neuropeptides can also have longer-term actions such as facilitating recovery of CPG function after loss of all modulatory input by decentralization [16•,56].

Several recent studies in *Aplysia* reveal the organizational ability of single neuropeptides to select the set of participating CPG neurons and/or their relative influence on circuit output [19••,20••,57••]. For instance a single neurally-released peptide (SCP) selects which circuit neurons are activated by an input pathway, via parallel coordinated actions within the feeding CPG [57••]. Specifically, stimulating axons in the esophageal nerve (EN) configures the feeding network into the egestion state, by exciting egestion-specific neurons (modulatory neuron B65, CPG neuron B20) and inhibiting an ingestion-specific CPG neuron (B40). This egestion state results largely from the EN axons releasing SCP, whose parallel and serial modulatory actions on these neurons enable the activation of the egestion-specific neurons and inhibition of ingestion-specific interneurons [57••] (Fig. 2a).

Another type of peptide-mediated circuit reorganization in the *Aplysia* feeding system is based on the long-lasting actions of two co-released peptides (Fig. 2b) [19••]. The ability of neurally-released peptides to trigger long-lasting circuit effects is not new [27,58–62]. What is new is that there are module-specific effects of the neuropeptides released by the projection neuron CBI-2 [19••]. Specifically, CBI-2-released peptides (CP2: cerebral peptide 2; FCAP: feeding-circuit activating peptide) directly enhance the activity of a protraction motor neuron (B48) and indirectly, via modulation of the ingestion CPG, inhibit the activity of a retractor motor neuron (B8) (e.g. motor neuron 1 vs. motor neuron 2; Fig. 2b). The authors propose the novel organizational principle that this peptidergic modulation is module-specific to optimize control of each neuron based on their pattern of synaptic input. In this case, B48 receives alternating excitation (during protraction) and inhibition (during retraction), so its direct modulation can separately influence its excitability during each phase (Fig. 2b). In contrast, B8 receives concurrent excitation and inhibition during both phases, and during different feeding patterns it tends to be active during either protraction or retraction (Fig. 2b). A phase specific B8 activity pattern is less likely to be supported by direct modulation of B8, as such modulation would likely influence its excitability during both phases. Both modes of synaptic regulation (concurrent vs alternating excitation and inhibition) are established in many different motor systems, but it remains to be determined whether direct vs. indirect modulation is module-specific as in the *Aplysia* ingestion motor program.

Distinct CPG outputs can also result from changes upstream to the CPG. In the crab STNS, two different extrinsic inputs (VCNs, POC neurons) trigger long-lasting but distinct gastric mill (chewing) rhythms by coactivating the same projection neurons (MCN1, CPN2) to drive the gastric mill CPG in the stomatogastric ganglion (STG) [27,63,64•]. The persistent influence of the POC neurons on these projection neurons results from its release of the tachykinin-related peptide CabTRP Ia [27]. The core rhythm generator underlying both gastric mill rhythms is composed of the same neurons, supporting the hypothesis that the distinct rhythms result entirely from differences in upstream modulation of the projection neurons [64•,65].

In the *Aplysia* feeding system, the same neuropeptide (ATRP: allatotropin-related peptide), via its release from an identified projection neuron (CBI-4) [66] and a motor neuron, provides feedforward compensation (Fig. 3a). This configuration results from ATRP coordinately altering the ingestion CPG (e.g. reducing protraction duration) and causing a compensatory strengthening of the associated protraction motor neurons (increasing their

firing rate) and protraction muscles (strengthening contractions and increasing relaxation rate) [20••]. The ATRP actions on the protraction muscles result from its corelease, with the neuropeptide myomodulin, from protraction motor neurons [20••]. Without the compensatory strengthening at the motor neuron and muscle levels, a shorter protraction duration would weaken the protraction muscle movements (Fig. 3a). Thus, ATRP released coordinately at central and peripheral sites stabilizes the strength of the ingestive behavior during cycle frequency changes that would otherwise compromise the behavior.

Feedforward compensation also underlies other coordinated movements, such as those mediating anticipatory postural adjustments, but the detailed cellular mechanisms underlying these latter events remain to be determined [67]. There are still few systems where network neurons are identified at multiple functional levels (e.g. projection, microcircuit, motor and sensory neurons), including their cotransmitter complements and synaptic actions. It therefore remains to be determined whether the coordinated actions of a single neuropeptide at multiple network levels, as in the *Aplysia* feeding system, is happenstance or is itself a new organizing principle. One additional system in which serial peptidergic actions are known to occur is the crab STNS, where the aforementioned POC neurons and the projection neuron MCN1 both release CabTRP Ia to trigger (POC neurons) and drive (MCN1) the gastric mill rhythm [27,59,68,69]. However, it is not known if there is a functional relationship between these separate CabTRP Ia sources analogous to those involving ATRP in *Aplysia*.

Two recent studies in the crab STNS also show that peptide modulation can directly modify CPG dynamics without changing the set of participating neurons or substantially changing the associated motor pattern. Instead, these peptide actions either stabilize the motor pattern [70••] or alter the CPG sensitivity to a parallel perturbation [13••,28••].

The peptide proctolin modulates the strength and short-term dynamics of the sole (inhibitory) CPG feedback synapse onto the pyloric pacemaker neurons in the crab STG [70••] (Fig. 3b). Further, although proctolin directly excites most pyloric circuit neurons [49], its presynaptic modulation of this feedback synapse alone is necessary and sufficient for the proctolin-mediated stabilization of the pyloric rhythm [70••,71]. This stability-enhancing feature occurred without a concomitant alteration of the pyloric motor pattern. Modulation of this same synapse by another neuropeptide (RPCH) was previously proposed to also stabilize the pyloric rhythm [72].

The Zhao et al. [70••] study used bath-applied proctolin, but their results likely reflect the influence of neurally-released proctolin insofar as the other, aforementioned proctolin influences on the pyloric CPG are equivalent to those mediated by the proctolinergic projection neuron MPN [58]. Interestingly, this equivalence occurs despite the fact that MPN contains cotransmitters, a priori suggesting that its influence on the pyloric CPG would result from the collective actions of these cotransmitters [49,59,73].

Peptide modulation can also modify circuit dynamics by influencing intrinsic currents, as shown in the crab gastric mill CPG [10,14] (Fig. 3c). The gastric mill rhythm is a two-phase motor pattern (protraction, retraction) whose core CPG includes reciprocal inhibition between the protractor neuron LG and retractor neuron Int1 [74,75]. During gastric mill rhythms driven by the projection neuron MCN1, the peptide hormone CCAP selectively albeit modestly increases the protraction phase duration [76]. This CCAP action results from its activating a single ionic current (I_{MI}) in the LG neuron, which is the same current activated in LG by the MCN1-released peptide CabTRP Ia (Fig. 3c) [13••].

Surprisingly, the dynamics of the MCN1- and CCAP-activated I_{MI} in LG are distinct during the gastric mill rhythm, because only MCN1-activated I_{MI} is weakened by feedback

inhibition during each protraction phase [13••,77]. Consequently, during protraction the declining influence of MCN1-activated I_{MI} is buoyed for a time by the small but maintained influence of CCAP-activated I_{MI} [13••]. Additionally, the unchanged retraction phase duration results not from a lack of CCAP influence but from CCAP-activated I_{MI} in LG preventing an increase in retraction duration [13••]. Without access to these mechanisms, inaccurate conclusions would likely have been drawn (e.g. the unchanged retraction duration resulted from CCAP being ineffective during this phase). Such inaccurate conclusions in turn would have compromised the subsequent insights attained with respect to the state-dependent influence of this CCAP action (see below).

Whereas the CCAP-mediated changes in the I_{MI} dynamics in LG only modestly alter the gastric mill rhythm in the isolated STNS, these altered dynamics gate out the influence of the aforementioned GPR sensory neuron [28••] (Fig. 3c). Using a dynamic clamp version of CCAP-activated I_{MI} to either add or subtract (in the presence of CCAP) I_{MI} in LG, DeLong and Nusbaum [28••] showed that the CCAP influence on LG is necessary and sufficient to eliminate this sensory feedback action. Thus, the CCAP modulation of an intrinsic current in a CPG neuron mediates the state-dependence of sensory regulation of circuit output.

Context-dependent modulation was also recently established for substance P in the mammalian respiratory CPG [12••]. Specifically, substance P excitation of the respiratory rhythm in the medullary pre-Bötzinger complex is modest during co-activation of noradrenergic and/or serotonergic input pathways, but its influence is pivotal when these parallel inputs are silent. Although the points of convergence in these separate actions remain to be determined, it is likely that the substance P influence becomes critical during sleep when the parallel aminergic pathways are weakly active or silent [12••].

Neuropeptide families

Neuropeptides differ from other metabotropic-acting transmitters in that they commonly are members of extended families, wherein family members often exhibit only small differences in their amino acid sequences [73,78–82]. Additionally, multiple peptide family members are often found in the same nervous system, and at least sometimes likely colocalize to the same neurons [83–85,86•]. In the STNS, separate application of different peptide family members have indistinguishable actions on the pyloric and gastric mill rhythms [73,87,88]. Interestingly, the influence of two different RFamide peptide families (FRFamides, FMRFamide) in *Aplysia* have distinct (but complementary) actions on the ingestion motor pattern [86•]. When the focus narrows to a single muscle target of the FRFamides, some family members have quantitatively equivalent actions while the dose-response curve for others is shifted [86•]. These observations raise questions, and hypotheses, regarding the purpose of colocalizing peptide family members.

Recent work in insects provides some insight by revealing that a single neuropeptide-activated GPCR can (a) differentially activate separate intracellular signaling systems when it is bound by different peptide family members [89,90] or (b) respond to only some family members [91•]. In the stable fly *Stomxys calcitrans*, an insect tachykinin (TK) receptor (STKR) has different relative influences on two separate signaling pathways when it is challenged with insect TK peptides containing an alanine vs. glycine residue near their C-terminal core motif [89]. In contrast, there is no such dichotomy for the *Drosophila* ortholog of STKR, called DTKR [90]. However, the other *Drosophila* tachykinin receptor (NKD) shares the STKR selectivity and responds only to the one *Drosophila* TK, of the six identified DTKs, with the alanine residue in its C-terminal core motif [91•].

Another degree of freedom available to peptide families results from the presence of different GPCRs in a single cell that can bind the same neuropeptide [42•]. The presence of

multiple receptors binding the same neuropeptide in a given nervous system, and particularly in a single neuron, provides the possibility that different peptide family members could have different affinities for each receptor type and thereby alter cellular physiology in distinct ways.

The above possibilities, however, are not sufficient to explain why different peptide family members have comparable actions on CPGs. It may be that some differences in amino acid sequences among peptide family members serve no distinct function and are simply "tolerated" by the system. Alternatively, it may be that different family members have overlapping but distinct actions, but the method of analysis (bath application) does not mimic the peptide actions when they are neurally-released and thus the differences between their actions are masked. For example, bath application might overwhelm the extracellular peptidases that normally limit peptide actions, and a given peptidase might not be equally effective at cleaving all peptide family members [68,92–94]. The persistent presence of the applied peptide during bath application, even at an appropriate concentration, might also enable the occurrence of relatively low affinity peptide-receptor binding events that do not occur during the more transient presence resulting from neural release.

Conclusions and Future Directions

Studies of peptidergic actions are providing new insights regarding the flexibility intrinsic to microcircuits. It is heartening that many of these new insights result from the neural release of peptides instead of their bath application. Bath application is a valuable tool for studying peptide modulation and in some situations is an appropriate and effective mimic of neural release, but this is not always the case (e.g. peptides are often coreleased with other transmitters). The consequences of cotransmission for microcircuits are established in only a few contexts, and promises to be a significant growth industry in the coming years. It is also likely that the number of degrees of freedom provided to microcircuits by peptidergic modulation will continue to grow, particularly as more effective tools become available to understand the influence of coreleased members of the same peptide family and the ability of peptide receptors to distinguish among them.

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activated, mitral cell-mediated gamma oscillation amplitude. The latter effect appeared to result from SST weakening the strength of the mitral cell excitation of granule cells at their dendro-dendritic synapses, which in turn weakens the subsequent granule cell inhibition of the mitral cell. The results support the hypothesis that SST regulates odor-mediated gamma oscillation amplitude and, hence, odor discrimination by its weakening mitral cell transmitter release (or possibly down-regulation of mitral cell GABA receptors) at the dendro-dendritic synapses between mitral- and granule cells. [PubMed: 20089895]

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ability of EN stimulation to organize the egestive state and suppress the ingestive state by implementing feedforward pathways. Specifically, SCP directly enhances the activity of B65 and B20 and inhibits B40 activity, while further facilitating B20 activity and suppressing B40 activity by its excitation of B65. [PubMed: 20181731]

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Highlights

- Neuropeptides contribute to the extensive functional flexibility of microcircuits
- Modulation of synaptic and intrinsic circuit properties alters circuit output
- Circuit output is also altered indirectly via modulation of circuit inputs
- Circuit modules can be differentially modulated by the same neuropeptide
- Functional consequences include stabilization at circuit and effector levels

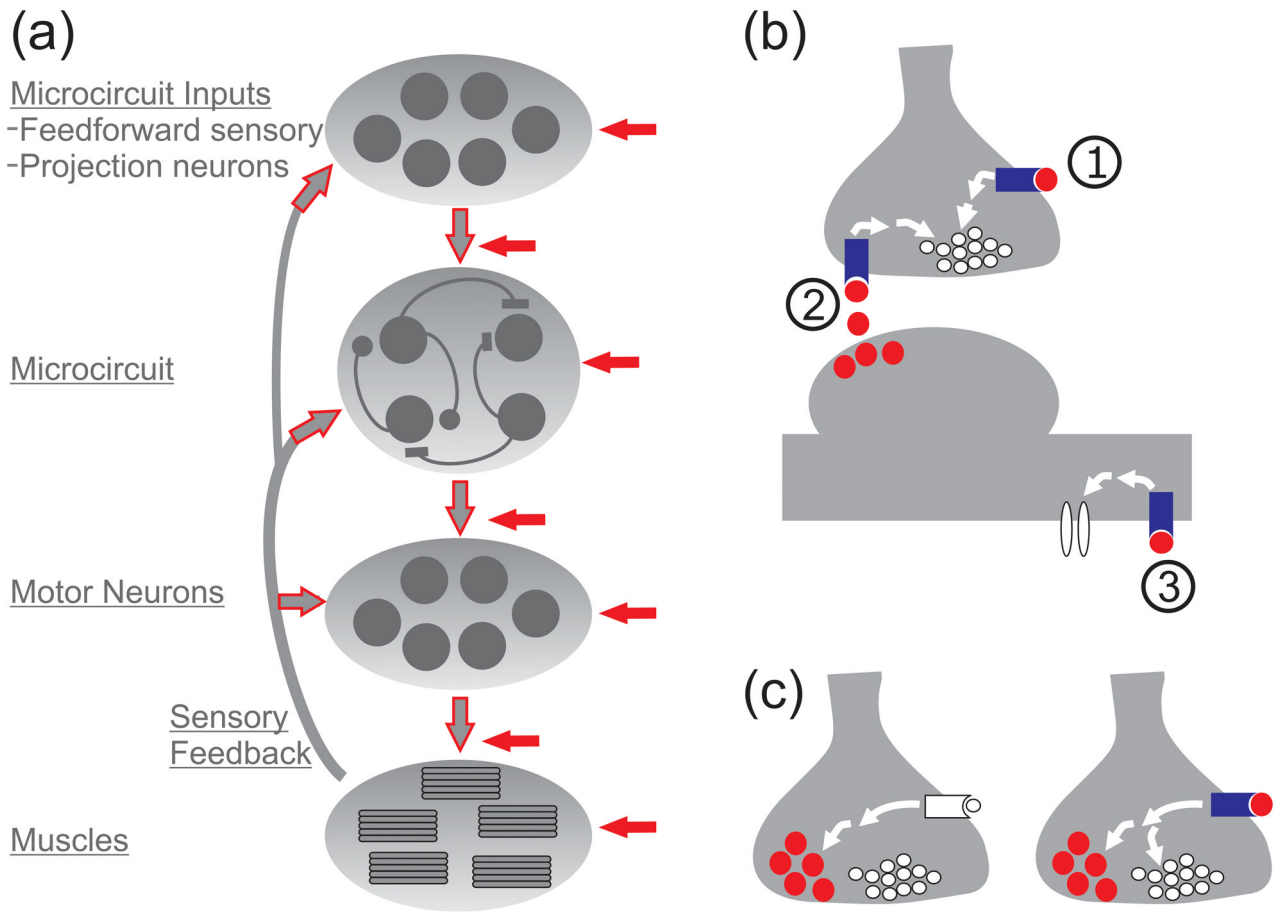

Figure 1.

Schematic illustrations showing that peptidergic modulation occurs at multiple sites. **(a)** Neuropeptides modulate the cellular and synaptic properties of neurons at every processing stage of microcircuits, including their feedforward (e.g. exteroceptors and projection neurons) and feedback (e.g. muscle sensory neurons) inputs, their effectors (e.g. motor neurons and muscles) and the microcircuit itself. These modulatory actions collectively alter the behavioral output of these systems. Red-filled arrows indicate sites of peptide modulation. Red outlined arrows indicate neurons that use neuropeptide transmitters. **(b)** Neuropeptides act presynaptically to alter transmitter release, commonly via second messenger systems (represented by white arrows) that are activated by neuropeptide binding to GPCRs. These neuropeptides (red circles) can be delivered from external inputs to a presynaptic site (site 1) or as retrograde messengers from the postsynaptic neuron (site 2). In addition to modulation of synaptic properties, neuropeptides can act via GPCRs to alter intrinsic currents (site 3). **(c)** *Left*, Metabotropic receptor activation can selectively regulate neuropeptide (red circles) release from a presynaptic terminal [39••]. *Right*, In addition, peptide binding to presynaptic GPCRs can regulate peptide release (and possibly small molecule transmitter release as well; white circles) [18••].

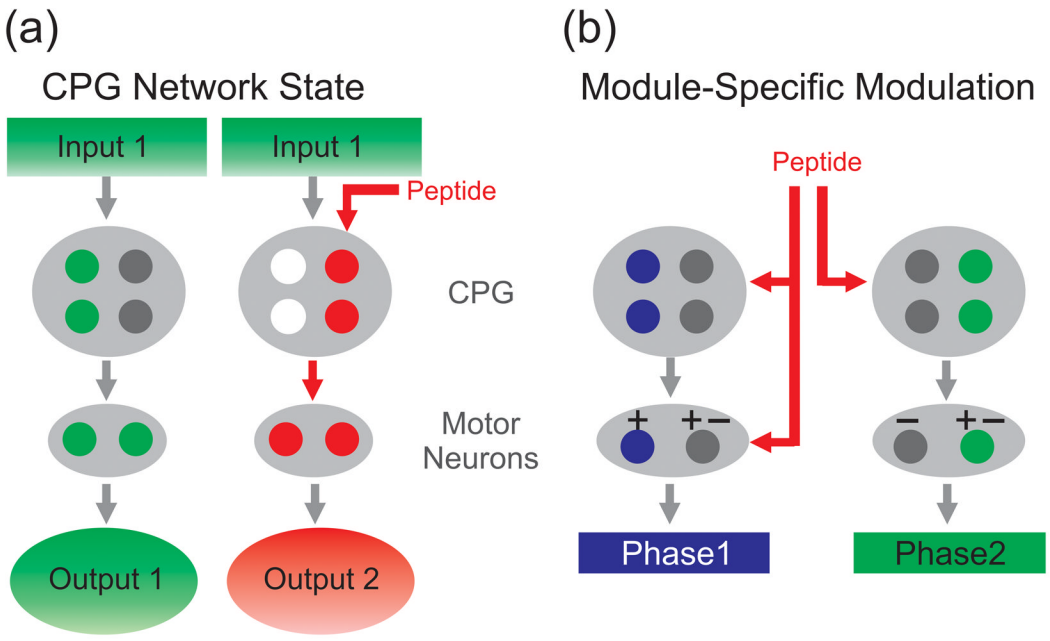

Figure 2.

Schematic illustrations representing peptidergic modulation of microcircuit output. **(a)** Peptidergic modulation of CPG neurons can select the set of active neurons and thereby establish a network state that determines the output elicited by a parallel input [57••]. *Left*, For example, Input 1 activates specific CPG neurons (green circles) to drive a particular motor neuron firing pattern (green) and elicit Output 1. *Right*, In contrast, in the presence of a particular neuropeptide, the same Input 1 now elicits a distinct output (Output 2) by activating different CPG neurons (red circles) while inhibiting the formerly active CPG neurons (white circles). To further ensure selection of Output 2, the peptide activates feedforward loops by enhancing excitatory and inhibitory synaptic actions from an "upstream" CPG neuron onto its "downstream" CPG targets (not shown; see [57••]). Symbols: Colored (red, green) circles, active neurons; grey circles, inactive neurons; white circles, inhibited by peptide input. **(b)** Peptidergic modulation can be module-specific [19••]. The left motor neuron receives alternating excitation (Phase 1) and inhibition (Phase 2) from the CPG during a two-phase rhythmic motor pattern. The activity of this motor neuron is directly modulated by a peptidergic input (red). The right motor neuron receives concurrent excitation and inhibition from the CPG during both phases of the rhythm. This motor neuron is indirectly modulated by the peptidergic modulation of CPG neurons. Symbols: +, excitation; −, inhibition; colored (blue, green) circles, active neurons; grey circles, inactive neurons.

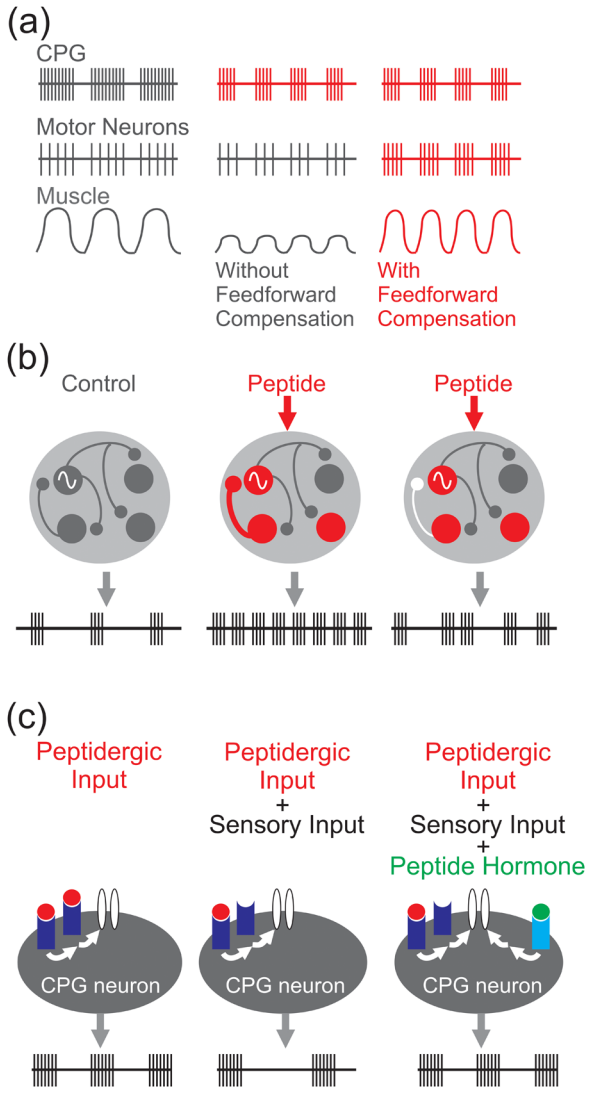

Figure 3.

Schematic illustrations of the functional consequences of neuropeptide modulation. **(a)** *Left, Middle*: Peptidergic modulation (red) solely to the CPG increases the rhythm frequency, shortening the duration of CPG neuron activity during each cycle. This in turn causes fewer motor neuron action potentials per cycle and weaker muscle contractions. *Right,* The addition of peripheral modulation by the same peptide (released by a motor neuron) provides feedforward compensation. In this latter case, there is a faster motor neuron firing frequency and a strengthened muscle response to motor neuron input, maintaining behaviorally-appropriate muscle contractions despite the shorter duration of this phase of the motor pattern [20••]. **(b)** Peptidergic modulation of a CPG feedback synapse is necessary for reducing the variability in the cycle duration during rhythmic motor activity [70••,71]. *Left, Middle*: Peptidergic modulation (red) increases the frequency of rhythmic output relative to control. *Right,* When the peptide-enhanced feedback synapse is selectively suppressed (white synapse), the peptide modulated intrinsic properties of circuit neurons maintain the faster rhythm, but the cycle duration is more variable. Sine wave indicates endogenously oscillatory pacemaker neuron. **(c)** Peptidergic modulation gates out sensory feedback [39••]. *Left,* A modulatory input uses peptide transmitter (red circle) binding to its receptor (blue) to

activate an intrinsic current in a CPG neuron. This modulation helps generate a particular rhythmic motor pattern in the isolated nervous system. *Middle,* The inclusion of sensory feedback decreases the release of this peptide via presynaptic inhibition, slowing activation of the intrinsic current and thereby slowing the rhythm. *Right,* Modulation by a peptide hormone (green circle) gates out the influence of the sensory input, stabilizing the motor pattern and decreasing its sensitivity to perturbation. This is accomplished by the actions of the peptide hormone converging onto the same intrinsic current activated by the peptidergic input in the circuit neuron, albeit via a different receptor (Iight blue) [13••,28••].