# Actions of identified neuromodulatory neurons in a simple motor system

Paul S. Katz and Ronald M. Harris-Warrick

*Recent work on neurons that release slow neuromodulators has revealed important generalities about the roles played by neuromodulation in motor systems. Activity of these cells can affect the cellular and synaptic properties of central pattern generating circuits, orchestrating new variations of motor patterns and sometimes coordinating their outputs with other motor patterns. Many modulatory neurons use multiple transmitters to evoke both fast and slow synaptic responses of various types in different target cells. Some modulatory cells can have a mediating as well as a modulating role, simultaneously acting as sensory neurons or components of another pattern generating circuit.*

*Paul S. Katz is at the Department of Biochemistry, Brandeis University, Waltham, MA 02254-9110, USA and Ronald M. Harris-Warrick is at the Section of Neurobiology and Behavior, Cornell University, Ithaca, NY 14853-2702, USA.*

Central pattern generators (CPGs) are networks of neurons that generate the timing and phasing cues for simple rhythmic movements[1]. It was initially believed that the output of a CPG was strictly determined by its pattern of synaptic connectivity. However, recent work in the crustacean stomatogastric nervous system and other systems has shown that CPGs are considerably more flexible than was originally thought. According to the 'polymorphic network' hypothesis of Getting and Dekin[2], a CPG is a single anatomically defined network which consists of a library of components that can be configured in different ways to form a number of different functional circuits. Neuromodulators such as monoamines and peptides play important instructive roles in determining the moment-to-moment output of a CPG by altering the synaptic strengths and intrinsic cellular properties of the circuit neurons.

Most experimental demonstrations of the effects of monoamines and peptides on CPG flexibility have used bath application[3–5]. To determine the physiological roles of these compounds, researchers are now seeking the neurons that normally release them and comparing the actions of these neuronally released and bath-applied modulators. Once the modulatory neurons are identified, researchers can also study the conditions that activate them, to provide a better understanding of the behavioral context for the actions of neuromodulators.

## The crustacean stomatogastric system

The stomatogastric nervous system of decapod crustaceans is one of the best described systems for the study of rhythmic motor pattern generation and modulation[6]. It consists of four small ganglia which contain the CPG networks controlling the movements of the three regions of the animal's foregut – the pylorus, the gastric mill, and the cardiac sac (Fig. 1A). The neurons forming the circuits for the gastric mill and pyloric CPGs are located in the stomatogastric ganglion, which contains 30 individually identifiable neurons. The synaptic organization and cellular components of these two circuits are largely known (Fig. 1B), allowing detailed studies of the modulation of the CPG neurons and synapses. A third, less studied CPG network, with components distributed among the different ganglia, controls the cardiac sac.

When the stomatogastric nervous system is removed from the animal, the circuits of the stomatogastric ganglion continue to generate rhythmic patterns of neural discharges that underlie motor programs. We will refer to these neuronal firing patterns as rhythmic motor patterns or simply rhythms. The gastric mill and pyloric rhythms cease when the stomatogastric ganglion is isolated from the other three ganglia in the stomatogastric nervous system (two commissural ganglia and the single esophageal ganglion). These other ganglia provide modulatory inputs to the stomatogastric ganglion that are essential for the production of rhythmic motor patterns by the CPG circuits in the stomatogastric ganglion. Researchers have identified 13 different neurotransmitters that can initiate or modulate the pyloric and/or gastric mill rhythm when bath-applied to the isolated ganglion[5,7]. Some of these substances normally reach the stomatogastric ganglion as circulating hormones[8], while others are present in sensory neurons or neurons located in the other ganglia that project to the stomatogastric ganglion.

In this review, we focus our discussion on five identified neurons that provide modulatory input to the stomatogastric ganglion. The locations of these neurons in relation to an idealized decapod foregut are shown in Fig. 1A, and their characteristics are summarized in Table I. There are two inferior ventricular nerve (IVN) neurons, two pyloric suppressors (PS), a single anterior pyloric modulator (APM), two modulatory proctolin-containing neurons (MPN), and four gastropyloric receptor (GPR) cells. These cells have been studied in different decapod species. Table I lists the primary genus studied for each cell. The distribution of each cell in the different decapod genera is shown in the inset to Fig. 1A.

The extent of experimental knowledge of the stomatogastric nervous system can seem overwhelming to those not familiar with it. Thus, we have attempted to extract general principles for the actions of modulatory neurons that may be applicable to other systems. These principles form the italicized headings of the following sections.

## Actions of modulatory neurons on motor patterns

When selectively stimulated, each of the five modulatory neurons induces unique changes in the stomatogastric motor patterns (Fig. 2). Activity in these modulatory neurons thus provides the instructions for the generation of multiple motor patterns by



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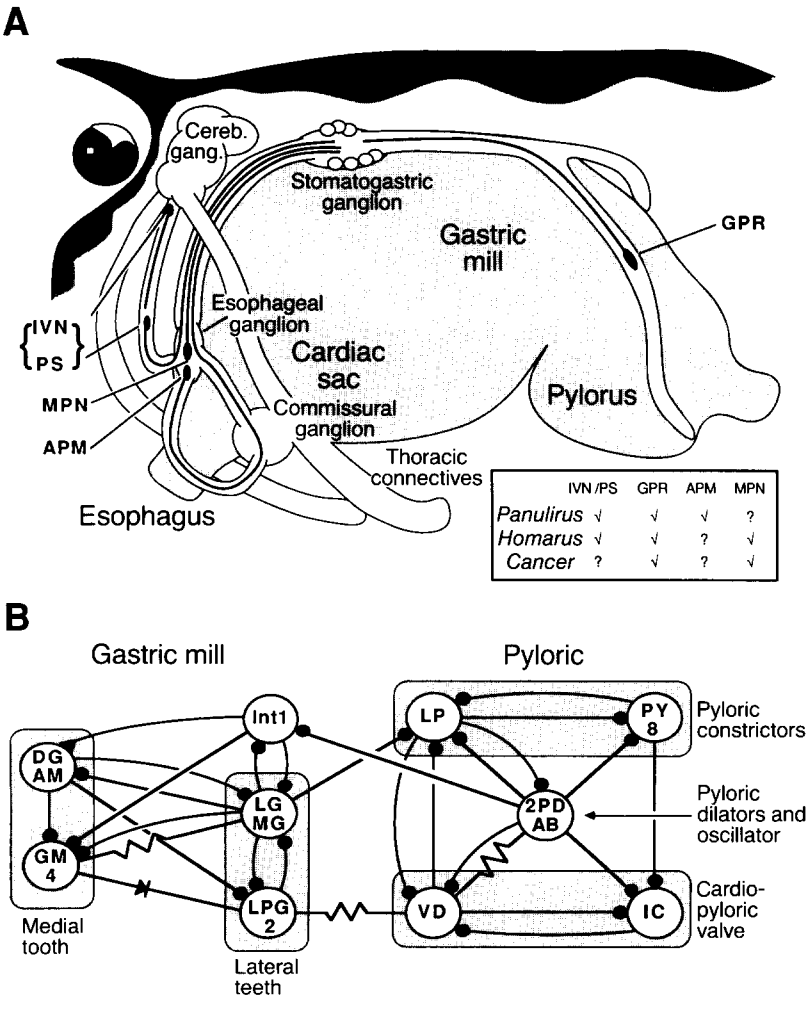


**Fig. 1. (A)** *Anatomy of the foregut and the stomatogastric system showing the locations of the five modulatory cell types. The modulatory neurons were identified in different species, but are shown here in an idealized decapod. For simplicity, only a single example is shown for each cell type. The inset shows representative decapod genera in which each cell is known to exist. The foregut of decapods is divided into three regions. Food enters the cardiac sac from the esophagus. Slow, irregular rhythmic movements of the cardiac sac move the food towards the gastric mill. The gastric mill consists of three internal teeth that macerate the food before it travels to the pylorus. In the pylorus, a series of brushes and valves filters the food, allowing fine particles to pass out of the foregut and into the midgut. Abbreviations: APM, anterior pyloric modulator; Cereb. gang., cerebral ganglion; GPR, gastropyloric receptor; IVN, inferior ventricular nerve cell; MPN, modulatory proctolin-containing neuron; PS, pyloric suppressor. (Note that IVN and PS are homologous neurons and are thus not found in the same species. PS is found in* Homarus *and IVN is found in* Panulirus.*)* **(B)** *The simplified synaptic connectivity diagrams for the pyloric and gastric mill CPG circuits in the stomatogastric ganglion of the spiny lobster,* Panulirus interruptus[6]. *Circles connote inhibitory synapses, triangles symbolize excitatory synapses, resistors show electrical coupling, and diodes signify rectifying electrical connections. For simplicity, some electrically coupled cell groups have been combined. Multiple cells are indicated by a number. Subsets of neurons have been grouped in shaded boxes that indicate their function in the control of the foregut. There are some differences between this circuit and the connectivity found in other species.*

each of the neuronal networks in the stomatogastric ganglion.

*Modulatory neurons affect multiple CPGs.* All five modulatory neurons alter the activity of the pyloric rhythm[9,11,12,16,17,19,22,24,25,30] (Fig. 2). In addition, two of the neurons, APM[27,28] and IVN[12], also affect the gastric mill rhythm, and have interactions with the cardiac sac rhythm. Another modulatory cell, GPR[21,23], modulates the activity of gastric mill neurons when the gastric mill rhythm is inactive, although its effects on the ongoing gastric mill rhythm have not been measured. Thus, the effect of a modulatory neuron is not limited to a single CPG.

*Modulatory neurons alter both the cycle frequency and the phasing of motor patterns.* The most detailed quantitative studies of the effects of the five modulatory neurons have been on the pyloric rhythm (Fig. 2). APM, GPR, IVN and MPN all activate or enhance the cycle frequency of the pyloric rhythm[11,12,19,21,22,24,25,30] (Fig. 2A–D). In contrast, PS, when strongly stimulated, inhibits the pyloric rhythm[16,17] (Fig. 2E). In addition to changing the cycle frequency, all of the modulatory neurons alter the phase relations of the individual pyloric neurons, allowing them to fire at a different point in the cycle or for a different fraction of the cycle (Fig. 3). This is the neural correlate of a modified behavior. Two modulatory cells, APM[27,28] and IVN[12], also evoke phase changes in the gastric mill cycle.

The phase relations of the motor patterns elicited by a modulatory cell depend on its stimulation parameters. For example, small increases in the firing frequency of PS change the pyloric pattern from an altered triphasic pattern, to a biphasic pattern (Fig. 3D) and finally to a previously undescribed, biphasic pattern with only two active neurons[16] (Fig. 3E). These frequencies are within the natural firing patterns of the cells. Often an initial modulatory change in motor pattern is replaced with new patterns if the stimulation is prolonged, or after it is terminated. For example, high-frequency stimulation of some of the modulatory cells initially disrupts pyloric activity, but following the stimulation there is a prolonged enhancement of pyloric cycling (e.g. GPR[22] and IVN[12] in Fig. 2A). These time-dependent effects may reflect differential time-courses in the effects of multiple neurotransmitters used by the cells (see below).

## Physiological actions of modulatory cells on individual CPG neurons

Some modulatory cells alter the synaptic interactions between CPG neurons[25], allowing a quantitative 'rewiring' of the CPG circuit diagram. In addition, the modulatory cells change the rhythmic motor patterns by having direct effects on the individual CPG neurons.

*Modulatory cells alter the intrinsic properties of CPG neurons.* Four of the five modulatory cells, APM, GPR, IVN and MPN, induce rhythmic membrane potential oscillations with bursts of action potentials in the major pacemaker of the pyloric CPG, the PD/AB cell group[11,21,26,30]. In contrast, PS suppresses rhythmic pacemaker bursting by powerful inhibition of the PD cells[17]. This ability to enable or inhibit pacemaker bursting underlies the activation or suppression of rhythmic pyloric cycling by the modulatory neurons.

Non-rhythmic bistable firing is another important variable property of many stomatogastric neurons: when a cell is bistable, a brief excitatory stimulus has a prolonged effect, activating a silent cell to a depolarized plateau potential with high-frequency firing (Fig. 4). Three of the modulatory neurons (APM[26,28], IVN[11] and GPR[21,22]) can induce plateau potentials in certain stomatogastric neurons but not in

others. This drastically changes the behavior and phasing of these cells in the motor circuit. Modulatory neurons can also suppress plateau properties. While APM enhances the plateau potential properties in many pyloric and gastric neurons, it suppresses plateau production in one gastric mill neuron[27]. PS suppresses plateau potential capabilities in two pyloric neurons[17].

*Each modulatory neuron has multiple slow effects on different follower neurons.* For example, GPR modulates both gastric mill and pyloric neurons in a variety of ways, including slow excitation, slow inhibition, and the enhancement of rhythmic bursting and bistable plateau properties[21–23]. The other modulatory neurons evoke similarly varied responses in different follower cells, but the overall constellation of effects evoked by each modulatory cell is unique.

The duration of action of a single modulatory cell varies among its different follower cells. For example, intense stimulation of PS inhibits activity in the pyloric LP cell for up to 2 min but inhibits the PD cell for only 30 s[16]. The maximal duration of modulatory effects is different for each modulatory cell; while brief stimulation of most of these cells can alter pyloric neuronal activity for over 1 min, the responses to a similarly brief MPN stimulus only last up to 10 s[30] (Fig. 2).

*Modulatory neurons use multiple transmitters to evoke both fast and slow responses.* In addition to slow modulatory effects, all five modulatory neurons also evoke classical rapid synaptic responses. In at least some of these cases, the multiple responses arise from the use of co-transmitters[16–20,31]. The co-transmitters can sometimes have synergistic effects. For example, GPR contains both acetylcholine and serotonin[19,20]. GPR input to a gastric mill neuron (DG) evokes rapid nicotinic EPSPs (Fig. 4A) and endows DG with the ability to produce plateau potentials when the cell is depolarized above a critical threshold[21] (Fig. 4B,C). This latter neuromodulatory effect is not due to the release of acetylcholine by GPR (Fig. 4D), but is mimicked by pressure ejection of serotonin onto the cell (Kiehn, O., pers. commun.). Thus, the co-transmitters act in concert: serotonin induces plateau potential capability, while the co-released acetylcholine provides the depolarization needed to reach threshold for triggering the plateau.

The other modulatory cells also evoke both fast and slow synaptic responses. MPN[31] and PS[16–18] (Meyrand, P., pers. commun.) both contain multiple transmitters (Table I). IVN may also contain multiple transmitters because histamine, its only known trans-

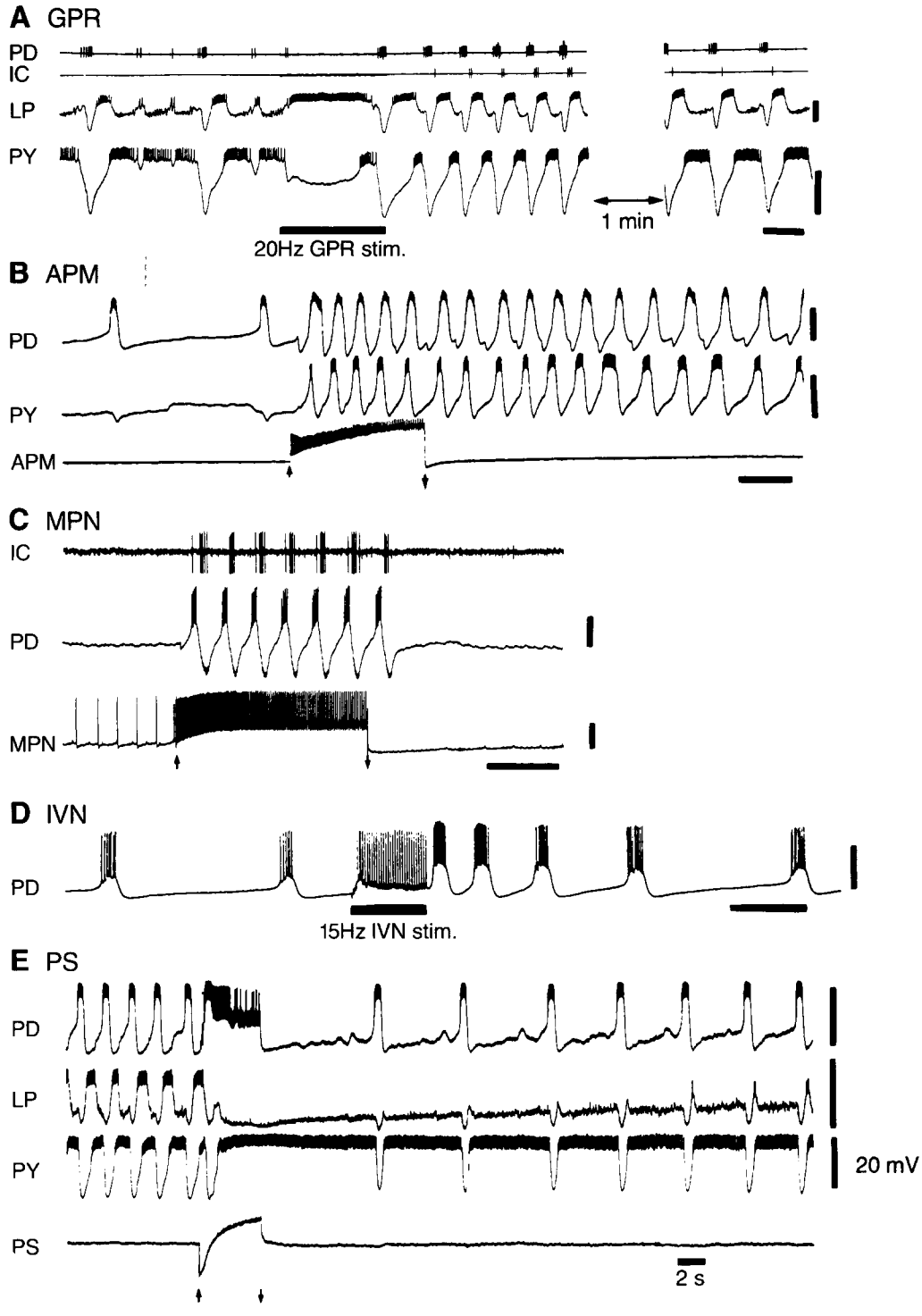


**Fig. 2.** *Typical effects of the modulatory cells on the pyloric motor pattern.* **(A)** *Gastropyloric receptor (GPR) stimulation (20 Hz, 5 s) causes an initial disruption of the slow pyloric pattern, followed by an increase in cycle frequency that lasts over 1 min*[22]. **(B)** *Anterior pyloric modulator (APM) stimulation (bottom trace) has a prolonged effect on the pyloric rhythm*[25]. **(C)** *Stimulation of the modulatory proctolin-containing neuron (MPN) can turn on the pyloric pattern, but its effects are short-lived*[30]. **(D)** *Inferior ventricular nerve cell (IVN) stimulation (15 Hz, 2 s) initially evokes EPSPs in PD, disrupting its activity. Following IVN stimulation, pyloric cycle frequency increases*[11]. **(E)** *Pyloric suppressor (PS) stimulation (bottom trace) also evokes EPSPs in PD, but causes a prolonged suppression of pyloric activity*[16].

**TABLE I.** Characteristics of neurons that provide modulatory input to the stomatogastric ganglion

| | Cell type | | | | |
|---|---|---|---|---|---|
| | **IVN** | **PS** | **GPR** | **APM** | **MPN** |
| **Known transmitters** | Histamine | Histamine, FMRFamide-like immuno-reactivity | Acetylcholine, serotonin | Acetylcholine | Proctolin, GABA-like immuno-reactivity |
| **CPG neurons affected** | Pyloric, gastric mill, cardiac sac | Pyloric | Pyloric, gastric mill | Pyloric, gastric mill, cardiac sac | Pyloric |
| **Activated by** | Cardiac sac CPG, pyloric stretch | Cardiac sac CPG | Mechano-receptor, endogenously rhythmic | Endogenously rhythmic | ? |
| **Primary genus studied** | *Panulirus* (spiny lobster) | *Homarus* (lobster) | *Cancer* (crab) | *Jasus* and *Palinurus* (cape and red lobsters) | *Cancer* (crab) |
| **Refs** | 9–15 | 16–18 | 19–23 | 24–28 | 29–31 |

Abbreviations: APM, anterior pyloric modulator; CPG, central pattern generator; GPR, gastropyloric receptor; IVN, inferior ventricular nerve cell; MPN, modulatory proctolin-containing neuron; PS, pyloric suppressor.

mitter, mimics just one of the several responses to IVN stimulation[11,13,15]. Some of the actions of APM are mimicked by acetylcholine[25], but other transmitters may also be involved.

*There are species differences in the phenotypes of the modulatory neurons.* Although each modulatory neuron has been studied primarily in one species, homologous cells have been identified in related decapods based on the anatomical projections they have in common. However, the physiological effects and the transmitter phenotypes of the homologues vary widely between species. For example, IVN, which is found in *Panulirus*, is homologous to the PS[17] neuron found in *Homarus*, but they have very different physiological effects: IVN enhances bursting in the PD/AB pacemaker group, while PS suppresses it. Both cells contain histamine[14] (Mulloney, B. and Hall, W., pers. commun.), but they may differ in other co-transmitters. Transmitter phenotypes of MPN and GPR vary between species; in crabs, MPN contains proctolin and displays GABA-like immunoreactivity[31], whereas the homologue of this cell in homarid lobsters shows GABA-like immunoreactivity, but lacks proctolin[32]. Similarly, in crabs GPR contains serotonin and acetylcholine[19,20], while the homologue in the spiny lobster does not contain serotonin[19], and that in *Homarus* contains serotonin[19] but also displays immunoreactivity to FMRFamide-like and CCK-like peptides (Meyrand, P. and Turrigiano, G., pers. commun.). Thus, these cells show a great deal of phylogenetic variability. It is not clear whether these differences are due to changes in functions of the neurons in the various species or whether they represent alternative mechanisms for fulfilling the same functions.

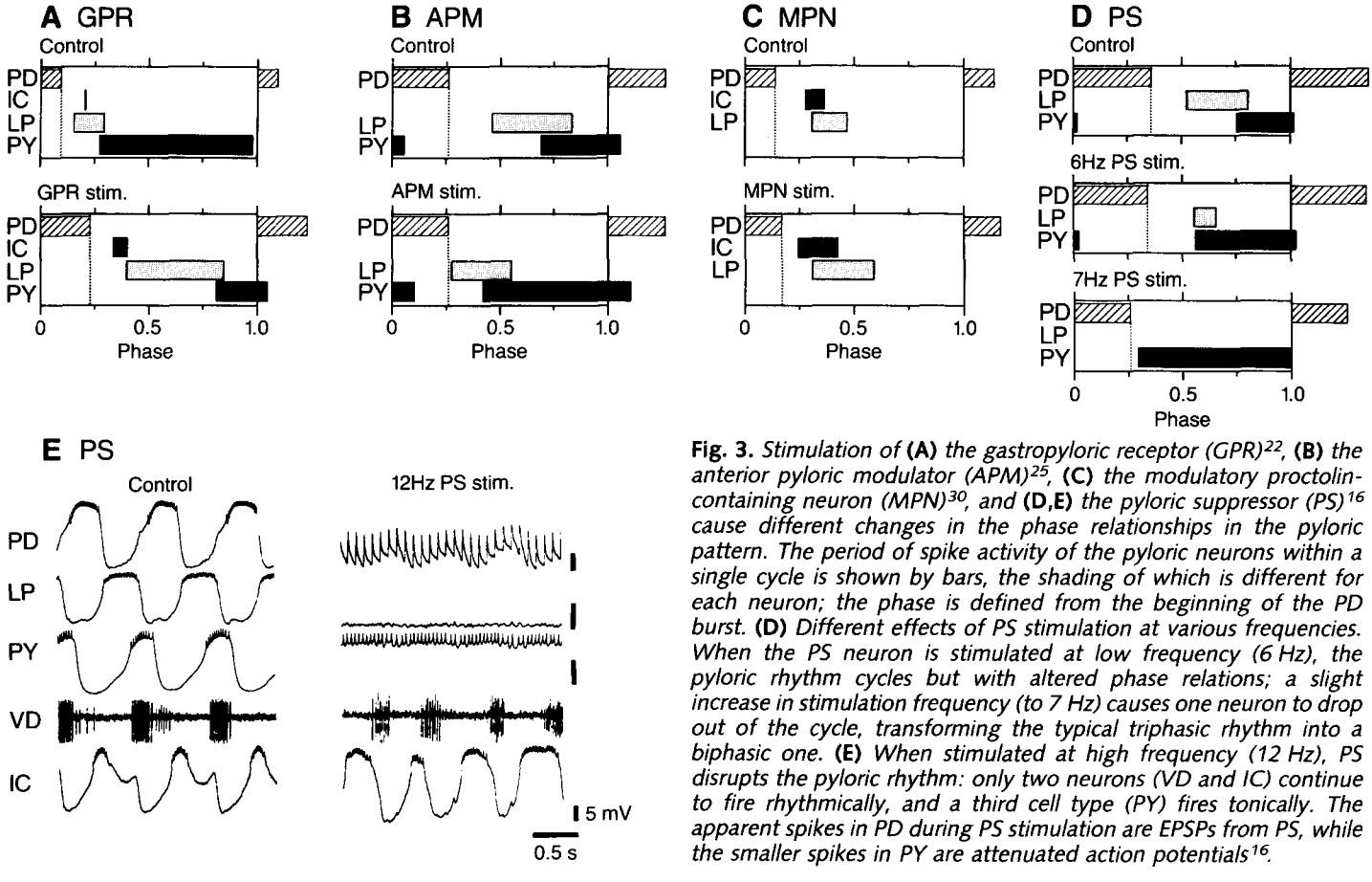


**Fig. 3.** *Stimulation of* **(A)** *the gastropyloric receptor (GPR)*[22], **(B)** *the anterior pyloric modulator (APM)*[25], **(C)** *the modulatory proctolin-containing neuron (MPN)*[30], *and* **(D,E)** *the pyloric suppressor (PS)*[16] *cause different changes in the phase relationships in the pyloric pattern. The period of spike activity of the pyloric neurons within a single cycle is shown by bars, the shading of which is different for each neuron; the phase is defined from the beginning of the PD burst.* **(D)** *Different effects of PS stimulation at various frequencies. When the PS neuron is stimulated at low frequency (6 Hz), the pyloric rhythm cycles but with altered phase relations; a slight increase in stimulation frequency (to 7 Hz) causes one neuron to drop out of the cycle, transforming the typical triphasic rhythm into a biphasic one.* **(E)** *When stimulated at high frequency (12 Hz), PS disrupts the pyloric rhythm: only two neurons (VD and IC) continue to fire rhythmically, and a third cell type (PY) fires tonically. The apparent spikes in PD during PS stimulation are EPSPs from PS, while the smaller spikes in PY are attenuated action potentials*[16].

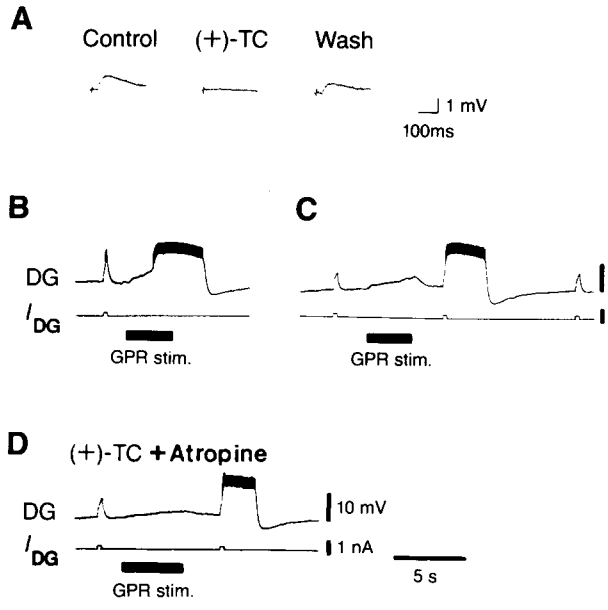


**Fig. 4.** *The gastropyloric receptor (GPR) has two separable effects on the DG neuron.* **(A)** *Individual GPR spikes evoke rapid synaptic potentials that are blocked by the nicotinic antagonist (+)-tubocurarine [(+)-TC].* **(B)** *When GPR is stimulated in trains at 20 Hz, the rapid EPSPs summate and trigger a plateau potential. A short current pulse ($I_{DG}$) prior to GPR stimulation causes DG to spike, but does not elicit a plateau potential.* **(C)** *When the EPSPs fail to depolarize DG sufficiently to evoke a plateau, a short current pulse following the GPR stimulus elicits a plateau potential. Prior to GPR stimulation, current injection cannot produce a plateau potential.* **(D)** *The nicotinic EPSPs are not necessary for the induction of plateau potentials. When the fast EPSPs are blocked with (+)-tubocurarine, GPR stimulation still induces plateau capability, which can be evoked by a brief depolarizing current pulse. Atropine is also present to preclude the participation of muscarinic receptors[21].*

## Functional and behavioral relevance

*Modulatory neurons can be sensory neurons or CPG components and can display endogenous rhythmic activity.* In order to understand the roles played by modulatory neurons in the stomatogastric nervous system, it is important to know when they are activated. Sensory input can activate some of these neurons either directly or indirectly. The IVN cells are activated indirectly by stretching the pyloric region[12]. The GPR cells are themselves primary sensory mechanoreceptors monitoring the tension of certain gastric mill muscles[20]. Proprioceptors are commonly thought only to have transient effects on motor output. However, GPR is an example of a sensory cell that also exerts direct and prolonged neuromodulatory actions on motor circuits.

Many of the modulatory neurons are rhythmically active. For example, both APM[25,28] and GPR[20] can produce endogenous rhythmic bursts of action potentials in the absence of external input, whereas IVN and PS are often rhythmically active because they appear to be components of the cardiac sac CPG[10]. Thus, instead of being tonically activated, these neurons release their transmitters in a rhythmic pattern.

*Modulatory neurons orchestrate new motor patterns.* Since many different neuromodulatory inputs can initiate the pyloric rhythm, it is unlikely that there is one single trigger to turn on the pyloric pattern. Conceivably, different activating stimuli could be funneled through different modulatory neurons to initiate somewhat different motor patterns. However, *in vivo* recordings from lobsters indicate that the pyloric pattern is almost always active[33]. Thus, a major role of neuromodulatory input is not so much to turn on the pyloric motor pattern, as to provide a long-lasting drive to maintain it. However, modulatory inputs may play an important role in initiating the

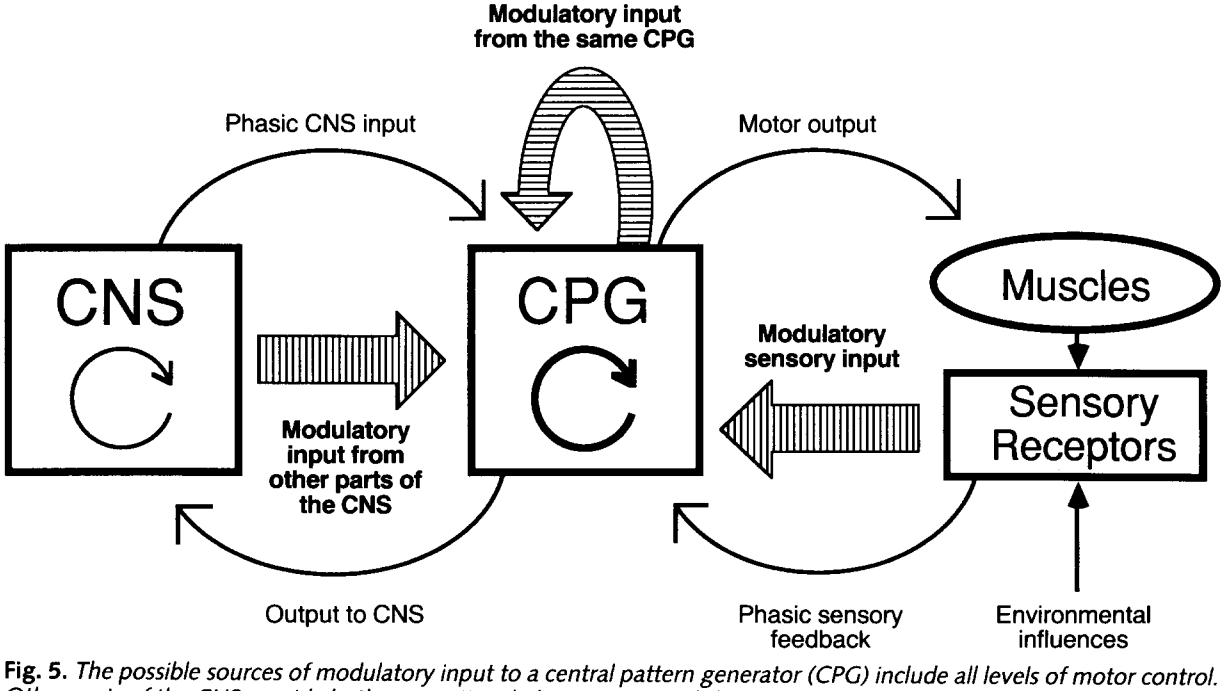


**Fig. 5.** *The possible sources of modulatory input to a central pattern generator (CPG) include all levels of motor control. Other parts of the CNS provide both conventional phasic inputs and slow modulatory inputs (left). These inputs include descending activating elements, hormonal influences and inputs from other CPGs. The CPG can contain neurons that evoke modulatory effects on other CPG components (middle). Sensory inputs provide not only conventional feedback acting on a cycle-by-cycle basis, but also slow modulatory effects that alter CPG function over many cycles (right).*

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gastric mill and cardiac sac motor patterns, which are often quiescent *in vivo*[8,33].

In addition to initiating and maintaining motor patterns, modulatory neurons can reconfigure the CPG circuits, altering the movements that are produced. For example, different modulatory inputs to the gastric mill network reconfigure it to produce at least two distinguishable behaviors. Spontaneous movements of the gastric mill in intact spiny lobsters have been observed with an endoscope and categorized into two major patterns of tooth movement, called the 'squeeze' and 'cut-and-grind' modes[34]. Stimulation of APM *in vitro* can activate the 'squeeze' motor pattern or change a 'cut-and-grind' pattern to a 'squeeze' pattern[27,28]. In part, this results from the selective activation and inactivation of plateau potential properties in different gastric mill neurons[27,28]. Injection of proctolin into the hemolymph, on the other hand, evokes the 'cut-and-grind' motor pattern[35].

*The modulatory neurons coordinate multiple motor patterns.* The three major motor patterns generated by the stomatogastric nervous system are usually described independently. However, *in vivo*, these behaviors need to be coordinated, and modulatory neurons could mediate this coordination. For instance, IVN stimulation causes the lateral gastric mill teeth to close in coordination with constriction of the pylorus[12]. Since IVN is a component of the cardiac sac CPG, its modulatory actions could serve to coordinate the outputs of the faster gastric mill and pyloric CPGs with that of the very slow cardiac sac CPG.

GPR is activated by movements of the gastric mill, but also affects the faster pyloric pattern[22]. Thus, in an intact animal, GPR may play a coordinating role by evoking periodic modulation of the pyloric pattern in-phase with the ongoing gastric mill rhythm.

*Neuromodulators configure conjoint CPGs.* CPG networks controlling different peripheral structures are typically thought to be separate and non-overlapping. However, recent work has shown that modulatory inputs can recruit neurons from one functional CPG into another. Hooper and Moulins[36] showed that stimulation of a sensory nerve causes one pyloric neuron temporarily to lose its regenerative oscillatory properties and to be recruited into the activated cardiac sac rhythm. Also, when the gastric mill is silent (which frequently occurs *in vivo*), GPR activity excites two gastric mill neurons to fire bursts of action potentials in-phase with the pyloric rhythm[23].

The peptide red pigment concentrating hormone (RPCH), which is present in axons that project to the stomatogastric ganglion[37,38], has even more drastic effects. Bath application of RPCH dramatically enhances weak synaptic interactions between the cardiac sac and gastric mill CPGs, fusing them into a single conjoint network that produces a combined motor pattern that had not been previously described[39]. Thus, neural circuits that operate independently of each other under some circumstances can share or exchange components under other circumstances.

## Concluding remarks

The 'polymorphic network' hypothesis[2] proposes that a single network can be reconfigured to form multiple functional circuits, each generating a unique motor pattern. Until recently, most of the data supporting this hypothesis came from experiments with bath-applied neuromodulators. The work described here shows that some of the same modulators can reconfigure the motor networks in the stomatogastric nervous system when released by identified neurons. Our understanding of the conditions under which these modulatory cells are activated is growing, and we can begin to put the cellular actions of neuromodulators in a behavioral perspective.

Similar results have been obtained in other systems. In *Aplysia*, several identified neurons modulate the motor networks underlying feeding and learning behaviors[40–42]. Some of these neurons contain multiple transmitters, including amines and peptides, and at least one is a primary sensory neuron[43,44]. In *Tritonia*, one of the component neurons in the swim CPG contains serotonin and modulates synaptic efficacy within the CPG during a swim episode[45]. Thus, modulatory neurons subserve other roles in addition to modulation and can be found at all levels of motor organization, from sensory receptors to CPG components to CPG-activating elements (Fig. 5); these are factors that future work in other systems should take into account.

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# Aspects of the structure of the $D_2$ dopamine receptor

Philip G. Strange

*Significant new information on the $D_2$ dopamine receptor has recently become available from a combination of protein chemical and molecular genetic analyses. Molecular genetic studies have shown the receptor to be a member of the family of receptors that are linked to G proteins and that have structures predicted to contain seven transmembrane domains. Two distinct species of $D_2$ dopamine receptor have been found which may differ in their coupling to G proteins; their distributions have been mapped at the nucleic acid level. The $D_2$ dopamine receptor has been purified from brain and anterior pituitary and characterized. Chemical modification of the brain receptor provides evidence for the importance of a carboxyl group that interacts with ligands at the receptor binding site. Here, Philip Strange discusses these points and proposes models of receptor–ligand interaction based on the conservation of several aspartic acid residues in receptors that bind cationic amines.*

*Philip G. Strange is at the Biological Laboratory, The University, Canterbury CT2 7NJ, UK.*

It is now well established that the actions of the neurotransmitter dopamine may be accounted for through its interaction with pharmacologically distinct $D_1$ and $D_2$ dopamine receptors. Several important actions of dopamine, including the central control of motor function and certain behaviours, the inhibition of pituitary hormone secretion and the modulation of cardiovascular function involve $D_2$ dopamine receptors; in addition, they are key sites of action of anti-parkinsonian and anti-schizophrenic drugs.

Understanding of the mechanism of action of the $D_2$ dopamine receptor is increasing: the receptor interacts with one or more G proteins (members of the $G_i/G_o$ family) to inhibit adenylate cyclase or open potassium ($K^+$) channels and possibly to attenuate calcium ($Ca^{2+}$) channel opening (reviewed in Ref. 1). The precise nature of the G proteins involved is not clear, neither is it clear whether a multiplicity of mechanisms of receptor action predicates multiple pharmacologically distinct receptor subtypes. Until recently, knowledge about the structure of the receptor has been sparse compared with that about the structure of other receptors[2]. This review summarizes some of the recent major advances in the structural analysis of the $D_2$ dopamine receptor.

## Purification of $D_2$ dopamine receptor protein

Important information can be obtained from the structural analysis of purified receptors but purification of the $D_2$ dopamine receptor has proved relatively difficult. The receptor can be solubilized with a good yield from brain and anterior pituitary using detergents[2], but the soluble receptor becomes unstable during purification and this has hindered attempts at purification. Nevertheless, under suitable conditions the soluble receptor can be successfully purified.

The $D_2$ dopamine receptor represents only about 0.002% of the protein in receptor-bearing tissues so that powerful affinity chromatography methods are required for purification of the receptor. Several affinity matrices have been developed based on the drugs haloperidol or spiperone linked to sepharose (reviewed in Refs 2,3), and recently three groups have reported purification of the $D_2$ dopamine receptor to apparent homogeneity from brain or pituitary[3–5] using these affinity matrices.

The receptor purified from brain runs as a major species on SDS polyacrylamide gel electrophoresis (SDS PAGE) with a molecular mass of 92–95 kDa[3,4]. Binding of [$^3$H]spiperone in the purified preparation has the correct pharmacological profile, and the specific activity is about 25% of the theoretical value for a protein of this molecular size. In our own study on the receptor from bovine brain[3], the purified material (molecular mass, 95 kDa) frequently runs as a doublet and this may reflect heterogeneous glycosylation (see below). The receptor purified from anterior pituitary runs as a major species on SDS PAGE of molecular mass 120 kDa[5]. Both species of receptor protein are glycoproteins as shown by their ability to bind to lectins. These differences in molecular mass are of added interest in the light of data



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