Behavioral/Systems/Cognitive

Hormonal Modulation of Sensorimotor Integration

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Neuronal circuits commonly receive simultaneous inputs from descending, ascending, and hormonal systems. Thus far, however, most such inputs have been studied individually to determine their influence on a given circuit. Here, we examine the integrated action of the hormone crustacean cardioactive peptide (CCAP) and the gastropyloric receptor (GPR) proprioceptor neuron on the biphasic gastric mill (chewing) rhythm driven by the projection neuron modulatory commissural neuron 1 (MCN1) in the isolated crab stomatogastric ganglion. In control saline, GPR stimulation selectively prolongs the gastric mill retractor phase, via presynaptic inhibition of MCN1. In the absence of GPR stimulation, CCAP does not alter retraction duration and modestly prolongs protraction. Here, we show, using computational modeling and dynamic-clamp manipulations, that the presence of CCAP weakens or eliminates the GPR effect on the gastric mill rhythm. This CCAP action results from its ability to activate the same modulator-activated conductance (G_{MI}) as MCN1 in the gastric mill circuit neuron lateral gastric (LG). Because GPR prolongs retraction by weakening MCN1 activation of G_{MI} in LG, the parallel G_{MI} activation by CCAP reduces the impact of GPR regulation of this conductance. The CCAP-activated G_{MI} thus counteracts the GPR-mediated decrease in the MCN1-activated G_{MI} in LG and reduces the GPR ability to regulate the gastric mill rhythm. Consequently, although CCAP neither changes retraction duration nor alters GPR inhibition of MCN1, its activation of a modulator-activated conductance in a pivotal downstream circuit neuron enables CCAP to weaken or eliminate sensory regulation of motor circuit output.

Introduction

Rhythmically active motor circuits generate multiple distinct motor patterns because of descending, ascending, and hormonal inputs (Jing and Weiss, 2005; Marder et al., 2005; Dickinson, 2006; Rossignol et al., 2006; Büschges et al., 2008; Doi and Ramirez, 2008). Despite the fact that parallel inputs to a circuit are likely commonly coactive (Dickinson, 2006; Marder and Bucher, 2007; Doi and Ramirez, 2008; Pearson, 2008), in most cases these inputs have been studied individually. Coactivating parallel inputs will not necessarily result in a simple summation of their individual actions because of circuit interactions and possibly interactions between these inputs (McLean and Sillar, 2004; Beenhakker et al., 2007; Blitz and Nusbaum, 2007; Barrière et al., 2008; Büschges et al., 2008).

We are addressing this issue by studying the influence of hormonal modulation on sensory feedback to a central pattern generator (CPG) circuit, using the isolated stomatogastric nervous system (STNS) of the crab *Cancer borealis* (Nusbaum and Beenhakker, 2002; Marder and Bucher, 2007). The STNS contains distinct but interacting CPGs whose outputs are regulated by projection neurons, sensory neurons, and hormones (Marder and Bucher, 2007). The gastric mill (chewing) CPG, located in the stomatogastric ganglion (STG), is driven by the projection neuron modulatory commissural neuron 1 (MCN1) (Nusbaum et al., 2001). This CPG includes the reciprocally inhibitory protractor phase neuron lateral gastric (LG) and retractor phase neuron interneuron 1 (Int1), plus the STG axon terminals of MCN1 ($MCN1_{STG}$) (Coleman et al., 1995; Bartos et al., 1999). MCN1 drives this rhythm via slow, peptidergic excitation of LG and fast, GABAergic excitation of Int1 (Wood et al., 2000; Stein et al., 2007). These actions occur during retraction, because LG inhibits $MCN1_{STG}$ during protraction (Coleman et al., 1995). MCN1 excites LG by activation of the modulator-activated inward conductance (G_{MI}) (DeLong et al., 2009a).

The MCN1-elicited gastric mill rhythm is altered by both the peptide hormone crustacean cardioactive peptide (CCAP) and the gastropyloric receptor (GPR) neuron (Beenhakker et al., 2005; Kirby and Nusbaum, 2007; DeLong et al., 2009a,b). Bath-applied CCAP selectively prolongs protraction, by providing a parallel activation of G_{MI} ($G_{MI\text{-}CCAP}$) in LG (DeLong et al., 2009a). GPR selectively prolongs retraction by presynaptically inhibiting $MCN1_{STG}$ and thereby reducing the rate at which MCN1-activated G_{MI} ($G_{MI\text{-}MCN1}$) builds up in LG (Beenhakker et al., 2005; DeLong et al., 2009b).

Here, we investigate how CCAP modulates GPR regulation of the MCN1–gastric mill rhythm. Our computational model predicts that adding $G_{MI\text{-}CCAP}$ to LG is sufficient to weaken GPR regulation of this rhythm, by reducing the impact of the GPR-induced reduction of $G_{MI\text{-}MCN1}$ in LG. Supporting this prediction, bath-applied CCAP weakened the GPR effect on this rhythm. Furthermore, dynamic-clamp-injected $G_{MI\text{-}CCAP}$ into LG mimicked this CCAP action, whereas dynamic-clamp subtraction of $G_{MI\text{-}CCAP}$ in LG during CCAP bath application eliminated the peptide regulation of this GPR action. These results illustrate that modulation of sensorimotor integration can result

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from a parallel activation of an ionic current in a downstream circuit neuron, without a direct action on either the sensory neuron or its synaptic target.

Materials and Methods

Animals. Male Jonah crabs (*C. borealis*) were obtained from commercial suppliers (Yankee Lobster; Marine Biological Laboratory). Crabs were housed in commercial tanks containing recirculating, aerated, artificial seawater (10–12°C). Before dissection, the crabs were cold-anesthetized by packing them in ice for at least 30 min. The foregut was then removed and maintained in chilled physiological saline while the STNS was dissected from it and pinned down in a saline-filled silicone elastomer-lined Petri dish (Sylgard 184; KR Anderson).

Solutions. The isolated STNS was maintained in *C. borealis* saline containing the following (in mM): 439 NaCl, 26 $MgCl_2$, 13 $CaCl_2$, 11 KCl, 10 Trizma base, and 5 maleic acid, pH 7.4–7.6. During experimentation, the preparation was continuously superfused (7–12 ml/min; 10–12°C) with *C. borealis* saline via a switching manifold, to enable fast solution changes. CCAP (Bachem) was diluted from stock solution into saline immediately before use.

Electrophysiology. All experiments were conducted using the isolated STNS from which the CoGs were removed by transecting the superior (*sons*) and inferior oesophageal nerves (*ions*) (see Fig. 1*A*). Intracellular and extracellular recordings of gastric mill neurons were made using routine methods for the STNS (Beenhakker and Nusbaum, 2004). Sharp glass microelectrodes (15–30 MΩ), filled with 0.6 M K_2SO_4 plus 10 mM KCl, were used for intracellular recordings. Intracellular recordings were made with Axoclamp 2 amplifiers (Molecular Devices), and intracellular current-clamp injections were performed in single electrode discontinuous current-clamp (DCC) mode with sampling rates of 2–5 kHz. To facilitate intracellular recordings, the STG was desheathed and visualized with light transmitted through a dark-field condenser (Nikon).

Each extracellular nerve recording was made using a pair of stainless-steel wire electrodes (reference and recording), the ends of which were pressed into the Sylgard-coated dish. A differential AC amplifier (model 1700; A-M Systems) amplified the voltage difference between the reference wire, placed in the bath, and the recording wire, placed near an individual nerve and isolated from the bath by petroleum jelly (Vaseline; Lab Safety Supply). This signal was then further amplified and filtered (model 410 amplifier; Brownlee Precision). Extracellular nerve stimulation was accomplished by placing the pair of wires used to record nerve activity into a stimulus isolation unit (SIU 5; Astro-Med/Grass Instruments) that was connected to a stimulator (model S88; Astro-Med/Grass Instruments).

To elicit the gastric mill rhythm in the isolated STG, MCN1 was selectively activated by tonic extracellular stimulation of one or both of the transected *ions* (10–15 Hz), on the STG side of the transection (see Fig. 1) (Coleman et al., 1995; Bartos and Nusbaum, 1997). The gastric mill protractor phase is defined as the duration of the LG burst, whereas the retractor phase is defined as the duration of the LG interburst/Int1 burst. Individual STNS neurons were identified by their axonal pathways, activity patterns, and interactions with other neurons (Weimann et al., 1991; Blitz et al., 1999; Beenhakker and Nusbaum, 2004).

GPR was selectively activated by extracellular stimulation of the gastropyloric nerve (*gpn*) (Katz et al., 1989; Beenhakker et al., 2005). Unless otherwise indicated, we stimulated GPR during the retractor phase of the gastric mill rhythm, to mimic its likely *in vivo* activity pattern (Katz et al., 1989), although tonic GPR stimulation has the same effect because GPR does not alter the gastric mill protractor phase (DeLong et al., 2009b). We used a stimulation frequency (5–15 Hz) within the frequency range exhibited by GPR in response to stretch of the muscles in which its dendrites are embedded (Katz et al., 1989; Birmingham et al., 1999). GPR stimulation was performed manually, by turning on the stimulator at the start of the retractor phase and terminating the stimulation before or immediately after the end of the retractor phase (i.e., LG burst onset). These stimulations were typically longer in duration than the control retractor phase duration because GPR stimulation prolongs this phase of the gastric mill rhythm (Beenhakker et al., 2005; DeLong et al., 2009b). In some experiments, we terminated GPR stimulation before the end of the retractor phase to ensure the continuation of the gastric mill rhythm. This approach was necessary because we used standardized MCN1 and GPR stimulation rates, and, under this condition, the balance between MCN1 excitation of LG and the GPR inhibition of $MCN1_{STG}$ sometimes favored the latter action. In those cases, LG could not escape from Int1 inhibition until GPR stimulation was terminated (Beenhakker et al., 2005; DeLong et al., 2009b). During these latter circumstances, we used an approximately threefold increase in retraction duration (30 s) as our cutoff duration, at which point we terminated the GPR stimulation.

Dynamic clamp. We used the dynamic clamp to inject an artificial version of a voltage-dependent ionic current (I_{MI}) into the LG neuron (Sharp et al., 1993; Bartos et al., 1999; Prinz et al., 2004; Beenhakker et al., 2005; DeLong et al., 2009a). The dynamic-clamp software uses the intracellularly recorded membrane potential of a biological neuron to calculate an artificial current (I_{dyn}) using a conductance [$g_{dyn}(t)$] that is numerically computed, as well as a predetermined reversal potential (E_{rev}). The artificial current is computed in real time, updated in each time step (0.2 ms) according to the new values of recorded membrane potential, and injected back into the biological neuron.

For these experiments, we used a version of the dynamic clamp developed in the Nadim Laboratory (Rutgers University, Newark, NJ) (available at http://stg.rutgers.edu/software/) to run on a personal computer (PC) running Windows XP and a NI PCI-6070-E data acquisition board (National Instruments). As above, all dynamic-clamp current injections were performed while recording in single-electrode, DCC mode (sampling rates, 2–5 kHz).

Data analysis. Data analysis was facilitated by a custom-written program (The Crab Analyzer) for Spike2 (Cambridge Electronic Design) that determines the activity levels and burst relationships of individual neurons (freely available at http://www.uni-ulm.de/~wstein/spike2/index.html). Unless otherwise stated, each data point in a data set was derived by determining the mean of 7–10 consecutive gastric mill-timed LG bursts, except during GPR stimulations when the average was taken across the duration of the GPR stimulation (1–5 cycles). In all experiments, the burst duration was defined as the duration (in seconds) between the onset of the first and last action potential in an impulse burst.

Data were collected onto a chart recorder (models MT 95000 and Everest; Astro-Med) and simultaneously onto a PC using data acquisition/analysis tools (Spike2; digitized at ~5 kHz). Figures were made from Spike2 files incorporated into Adobe Illustrator (Adobe). Statistical analyses were performed with Microsoft Excel (Microsoft) and SigmaStat 3.0 (SPSS). Comparisons of phase durations were made using the paired Student's *t* test unless otherwise indicated. For one data set, the repeated-measures ANOVA (RM-ANOVA) followed by the Student–Newman–Keuls (SNK) *post hoc* test was used to verify that, as previously shown, CCAP superfusion had no effect on the baseline retraction duration (DeLong et al., 2009a). In all experiments, the effect of each manipulation was reversible, and there was no significant difference between the premanipulation and postmanipulation groups. Data are expressed as the mean ± SE.

Computational model. To study the hormonal CCAP influence on the GPR regulation of the gastric mill rhythm, we modified an existing conductance-based computational model of the gastric mill circuit (Nadim et al., 1998; Beenhakker et al., 2005; DeLong et al., 2009a). Specifically, we retained from the previous versions of this model the LG, Int1, and MCN1 neurons having multiple compartments separated by an axial resistance, with each compartment possessing intrinsic and/or synaptic conductances (see Fig. 2*A*). We combined our previously published models that included GPR (Beenhakker et al., 2005) and CCAP (DeLong et al., 2009a), but we did not add or modify any synaptic or intrinsic conductances beyond those previously published (Tables 1, 2).

As published previously (Beenhakker et al., 2005), to model the GPR neuron we used a single-compartment cell with a passive leak current plus Hodgkin–Huxley-like voltage-dependent inward and outward currents to enable action potential generation. Consistent with physiological measurements (Beenhakker et al., 2005), this model neuron made an inhibitory synaptic connection onto a distal compartment of the MCN1 axon terminals (t0 compartment) (see Fig. 2*A*) (Nadim et al., 1998). To mimic the cumulative action of repeated GPR stimulations, the GPR

Table 1. GPR model parameters

	G_{max} (nS)	E_{rev} (mV)	p	q	m_{inf}	m_τ	h_{inf}	h_τ
Synapses								
GPR–MCN1	40	−80	—	—	$\frac{1}{1+e^{-(V+60)}}$	$2000+\frac{4000}{1+e^{V+60}}$	—	—
GPR intrinsic currents								
Leak	3	−68	N/A	N/A	N/A	N/A	N/A	N/A
Na	120	50	3	1	$\frac{1}{1+e^{-5(V+62)}}$	Inst	$\frac{1}{1+e^{5(V+64)}}$	$1+\frac{5}{1+e^{-0.24(V+64)}}$
K	36	−77.5	4	N/A	$\frac{1}{1+e^{-5(V+54)}}$	$8+\frac{20}{1+e^{0.24(V+54)}}$	N/A	N/A

Parameters used to incorporate GPR into an existing model of the gastric mill rhythm (Nadim et al., 1998; Beenhakker et al., 2005). GPR was modeled as a single-compartment cell with active and passive properties, and the slowly activating GPR–MCN1 synapse was added. Abbreviations: E_{rev}, reversal potential; G_{max}, conductance value at maximum activation; h_{inf}, steady-state inactivation curve; h_τ, inactivation time constant; Inst, instantaneous time constant; m_{inf}, steady-state activation curve; m_τ, activation time constant; N/A, not relevant to computation; p, integer power of the activation variable m; q, integer power of the inactivation variable h. Parameter names are derived from the nomenclature used in the Network modeling software, which was used to perform all simulations (http://stg.rutgers.edu/software/network.htm).

Table 2. Gastric mill rhythm model parameters for $I_{MI\text{-}CCAP}$

Current	G_{max}	E_{rev}	m_{inf}	m_τ	$m_{postinf}$	$m_{post\text{-}\tau}$	p
$I_{MI\text{-}CCAP}$	0.2	0	$\frac{1}{1+e^{-0.1(V_{LG}+50)}}$	50	N/A	N/A	1

The values used for $I_{MI\text{-}CCAP}$ in the computational model of the MCN1-elicited gastric mill rhythm (DeLong et al., 2009a). Abbreviations: $m_{postinf}$, Steady-state activation curve for the postsynaptic voltage dependence; $m_{post\text{-}\tau}$, activation time constant for the postsynaptic voltage dependence. See Table 1 for all other abbreviations. Parameter names are derived from the nomenclature used in the Network modeling software, which was used to perform all simulations (http://stg.rutgers.edu/software/network.htm).

synapse onto MCN1 was modeled as a slowly activating, slowly deactivating current (Beenhakker et al., 2005). The conductances and other parameters of these currents were chosen to mimic the behavior of the gastric mill circuit in the presence of GPR stimulation (Table 1).

Additionally, as published previously (DeLong et al., 2009a), we added to the LG neuron dendrite compartment an intrinsic (i.e., nonsynaptically activated) current ($I_{MI\text{-}CCAP}$) with the same voltage dependence as $I_{MI\text{-}MCN1}$ (Table 2) to mimic the effects of CCAP bath application on the biological system. This approach was based on the fact that CCAP and MCN1-released *C. borealis* tachykinin-related peptide Ia (CabTRP Ia) both excite LG by activating I_{MI} in this neuron (DeLong et al., 2009a).

Simulations were performed on a PC with Windows XP. We used the Network simulation software developed in the Nadim laboratory (http://stg.rutgers.edu/software/network.htm), which was run using the freely available CYGWIN Linux emulation software package. We used a fourth-order Runge–Kutta numerical integration method with time steps of 0.05 and 0.01 ms. Results were visualized by plotting outputted data points using the freely available Gnuplot software package (www.gnuplot.info).

It is important to note that the I_{MI} presentation in the model and dynamic-clamp figures represents different conventions. Specifically, the model output directly reports actual current flow in the model neuron and so uses the standard voltage-clamp convention. In contrast, the dynamic-clamp output represents the current injected into the neuron and hence uses the standard current-clamp convention. Consequently, I_{MI} is represented as an inward (downward trajectory) current in the model output figures but is represented as a depolarizing (upward trajectory) current injection in the dynamic-clamp output figures.

Results

CCAP and GPR prolong the gastric mill protractor and retractor phases, respectively, by regulating G_{MI} in the LG neuron

When manipulated separately, both CCAP and GPR influence the MCN1-elicited gastric mill rhythm (Fig. 1*B–D*). CCAP gains access to the STG as a circulating hormone (Christie et al., 1995; Li et al., 2003; Chen et al., 2009). Bath-applied CCAP (threshold, 10^{-10} M) causes a modest but consistent prolongation of the protractor phase during the MCN1–gastric mill rhythm (Fig. 1*B*) (Kirby and Nusbaum, 2007). This CCAP action results from its convergent activation of the modulator-activated inward conductance G_{MI} in LG with that of MCN1-released CabTRP Ia (DeLong et al., 2009a). $G_{MI\text{-}CCAP}$ sums with $G_{MI\text{-}MCN1}$ in LG, but they have different activation and deactivation trajectories during protraction because only $G_{MI\text{-}MCN1}$ is synaptically regulated by LG. Consequently, during protraction, $G_{MI\text{-}CCAP}$ maintains a relatively constant amplitude while $G_{MI\text{-}MCN1}$ decays because of the inhibitory synapse from LG to $MCN1_{STG}$ (Figs. 1*C*, 2). The maintained $G_{MI\text{-}CCAP}$ amplitude sums with the decaying $G_{MI\text{-}MCN1}$ to keep LG suprathreshold for a longer duration, prolonging its burst (DeLong et al., 2009a). By summing with $G_{MI\text{-}MCN1}$, $G_{MI\text{-}CCAP}$ also acts to prevent a change in the gastric mill retractor phase duration (DeLong et al., 2009a). This unchanged retraction duration occurs because the $G_{MI\text{-}CCAP}$ compensates for a reduced $G_{MI\text{-}MCN1}$ amplitude during retraction, which results from the $G_{MI\text{-}MCN1}$ decaying to a lower level during the prolonged protraction phase (DeLong et al., 2009a).

GPR is a bilaterally symmetric pair of muscle stretch-sensitive sensory neurons whose dendrites arborize in gastric mill protractor muscles (Katz et al., 1989). Hence, the GPRs are activated during retraction, when the protractor muscles are stretched (Katz et al., 1989; Katz and Harris-Warrick, 1989). GPR selectively prolongs the gastric mill retractor phase by its presynaptic inhibition of $MCN1_{STG}$ (Fig. 1*C*,*D*) (Beenhakker et al., 2005; DeLong et al., 2009b). Mechanistically, this GPR action appears to reduce the rate of CabTRP Ia release from MCN1, which slows the MCN1 activation of $G_{MI\text{-}MCN1}$ in LG (Beenhakker et al., 2005; DeLong et al., 2009b). This GPR action occurs without causing a parallel alteration in the MCN1 GABAergic excitation of Int1 (DeLong et al., 2009b). Consequently, GPR prolongs retraction by slowing the buildup of $G_{MI\text{-}MCN1}$ in LG and hence slowing the ability of LG to escape from Int1 inhibition and reach burst threshold (Figs. 1*D*, 2).

CCAP gates the GPR regulation of the gastric mill rhythm in a computational model

We investigated whether the presence of CCAP influenced the ability of GPR to regulate the gastric mill rhythm. We assessed this possibility because GPR regulates the gastric mill rhythm by influencing the ability of MCN1 to activate G_{MI} in LG, and CCAP directly activates G_{MI} in LG. To this end, we combined two pre-

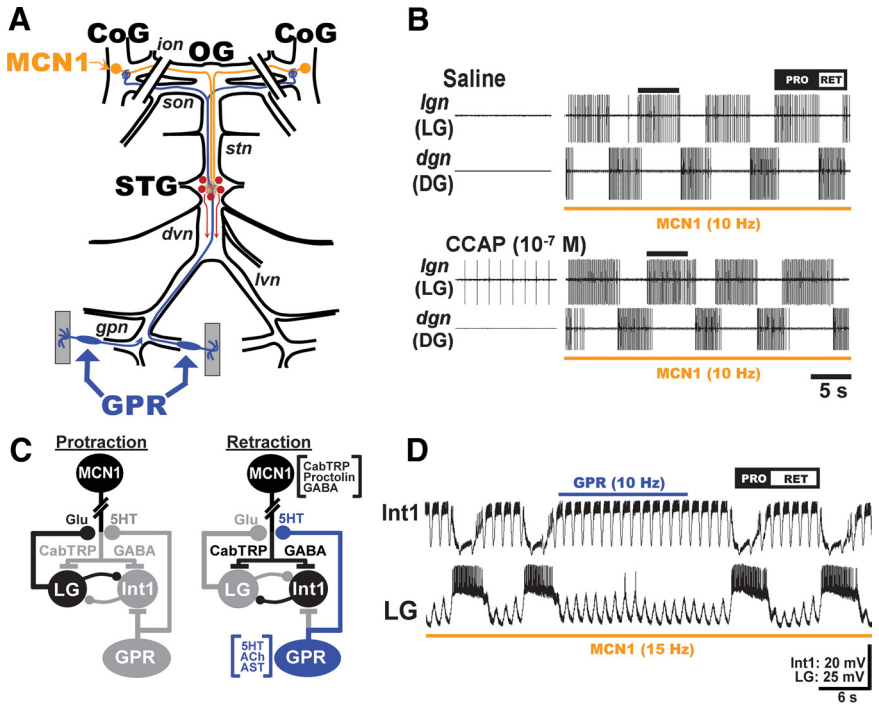

Figure 1. Schematics of the isolated STNS and the MCN1-elicited gastric mill circuit, as well as its regulation by CCAP and GPR. ***A***, In each CoG, there is a single copy of the projection neuron MCN1, which projects an axon through the *ion* and *stn* nerves to the STG. Each GPR projects an axon through the *lvn* and *dvn* nerves to arborize in the STG and continues through the *stn* and *son* nerves to innervate each CoG. The paired diagonal bars through the *sons* and *ions* represent the transection of these nerves at the start of each experiment. The gray rectangles represent protractor muscles in which the GPR dendrites arborize. These muscles were removed for the experiments in this paper. Abbreviations: Ganglia: CoG, commissural ganglion; OG, oesophageal ganglion. Nerves: *dvn*, dorsal ventricular nerve; *lvn*, lateral ventricular nerve; *stn*, stomatogastric nerve. ***B***, As shown by Kirby and Nusbaum (2007), bath-applied CCAP selectively prolongs the protractor phase of the MCN1-elicited gastric mill rhythm. Note that CCAP did not activate the gastric mill rhythm before MCN1 stimulation. Protraction (PRO) phase is represented by the LG protractor neuron activity. Retraction (RET) phase is represented by the dorsal gastric (DG) retractor neuron activity. The bar on top of second LG burst in each panel represents the LG burst duration in saline, to show that the LG burst is prolonged by CCAP. This panel was reproduced from the study by DeLong et al. (2009a). ***C***, Gastric mill CPG circuit schematics during each phase of the gastric mill rhythm. The paired diagonal bars through MCN1 axon represent additional distance between CoG and STG. All synapses shown are located in the STG. The gray somata and synapses represent neurons/synapses that are inactive during the indicated phase of the gastric mill rhythm. Synapses drawn on somata or axons actually occur on small branches in the STG neuropil. Transmitters in brackets next to MCN1 and GPR somata are their identified cotransmitters. Note that MCN1 uses only CabTRP Ia to excite LG and only GABA to excite Int1, whereas GPR uses only 5-HT to inhibit $MCN1_{STG}$ (Wood et al., 2000; Stein et al., 2007; DeLong et al., 2009b). LG and Int1 are both glutamatergic (Marder, 1987; Saideman et al., 2007a). Transmitter abbreviations: 5-HT, 5-hydroxytryptamine (serotonin); Glu, glutamate. Symbols: Filled circles, Synaptic inhibition; T-bars, synaptic excitation. ***D***, GPR stimulation selectively prolongs the gastric mill retractor phase. Note that the duration of the retractor phase during GPR stimulation is longer than the same phase in the cycles immediately before and after GPR stimulation (Beenhakker et al., 2005). The fast rhythmic action potential bursts in Int1 and the associated fast rhythmic subthreshold LG oscillations represent the influence of the pyloric circuit on the gastric mill CPG (Bartos et al., 1999). The rhythmic subthreshold depolarizations in LG result from the rhythmic removal of Int1-mediated inhibition and the consequent unmasking of MCN1-mediated excitation. ***A***, ***C***, and ***D*** are reproduced from the study by DeLong et al. (2009b).

viously published versions of our computational model of the MCN1–gastric mill circuit. Specifically, we modified our existing model of the gastric mill CPG plus GPR (Beenhakker et al., 2005; DeLong et al., 2009b) by adding $G_{MI\text{-}CCAP}$ to LG, using previously published parameters (Tables 1, 2; Fig. 2*A*) (DeLong et al., 2009a). As shown previously, in the version of the model in which $G_{MI\text{-}CCAP}$ was absent from LG, GPR stimulation prolonged the retractor phase (pre-GPR, 7.5 s; during GPR, 34.4 s) without altering the protractor phase (pre-GPR, 7.7 s; during GPR, 7.4 s) (Fig. 2*A*) (Beenhakker et al., 2005).

Adding $G_{MI\text{-}CCAP}$ to LG in our model, in the absence of GPR activation, modestly prolonged the protractor phase (control, 7.7 s; CCAP, 10.7 s) without altering the retractor phase duration (control, 7.5 s; CCAP, 7.1 s), as occurs in the biological STG (Fig. 2*B*) (Kirby and Nusbaum, 2007). When GPR was stimulated while $G_{MI\text{-}CCAP}$ was present in LG, the GPR prolongation of retraction was reduced by >10-fold, from >400 to ~40% of the control value (pre-GPR, 7.1 s; during GPR, 10.0 s), whereas the protractor phase duration remained unchanged (pre-GPR, 10.7 s; during GPR, 10.6 s) (Fig. 2*C*).

The apparent reason for the weakened GPR effect in the model that included $G_{MI\text{-}CCAP}$ in LG was that the addition of this conductance provided an alternative source of G_{MI} that was not subject to GPR regulation (Figs. 2*C*, 3). As stated above, GPR regulates $G_{MI\text{-}MCN1}$ in LG by inhibiting $MCN1_{STG}$ (Beenhakker et al., 2005; DeLong et al., 2009b), but it has no influence on $G_{MI\text{-}CCAP}$ in LG. Despite this distinction in their synaptic regulation, when both G_{MI} components were present, they summed, and, hence, so did the resulting $I_{MI\text{-}MCN1}$ and $I_{MI\text{-}CCAP}$ (DeLong et al., 2009a). For example, in the absence of GPR stimulation, the total maximal I_{MI} amplitude in LG, which occurred during the pyloric rhythm-timed depolarization immediately preceding LG burst onset, was changed by <5% by the presence of CCAP (CCAP absent, −21.2 pA; CCAP present, −20.2 pA). Thus, $I_{MI\text{-}MCN1}$ and $I_{MI\text{-}CCAP}$ summed to the same level as $I_{MI\text{-}MCN1}$ in the absence of CCAP (DeLong et al., 2009a).

The fact that $I_{MI\text{-}MCN1}$ and $I_{MI\text{-}CCAP}$ summed suggested that, in the presence of CCAP, the fraction of the total I_{MI} influenced by GPR would be smaller, and, hence, GPR would have less impact on the ability of this current to depolarize LG and enable it to reach burst threshold. Supporting this hypothesis, in our model the GPR stimulation in the presence of CCAP delayed LG burst onset only slightly relative to the preceding and subsequent cycles (Fig. 2*C*), and the maximal I_{MI} amplitude immediately before LG burst onset (−19.5 pA) was similar to cycles in which GPR was not stimulated (−20.2 nA). This maximal I_{MI} was the summed result of its activation by MCN1 plus CCAP ($I_{MI\text{-}MCN1}$, −16.5 pA; $I_{MI\text{-}CCAP}$, −3.0 pA).

The corollary to the preceding hypothesis is that, during GPR stimulation, at any given time point during the buildup of I_{MI} during retraction the maximal total I_{MI} amplitude should be larger in the presence of CCAP. Indeed, whereas the maximal I_{MI} was −19.5 nA at the end of retraction (10 s after retraction onset) during GPR stimulation in the presence of CCAP, when measured during GPR stimulation in the absence of CCAP at the same time point its amplitude (contributed entirely by $I_{MI\text{-}MCN1}$) was smaller (−17.2 pA). Additionally, this amplitude was smaller than the maximal I_{MI} needed for LG to reach burst threshold. Consequently, under these conditions, LG burst onset did not occur for another ~25 s (Figs. 2, 3).

These events are more explicitly compared in Figure 3, which presents the G_{MI} levels during one model gastric mill cycle in which GPR was stimulated, in the absence versus presence of $G_{MI\text{-}CCAP}$. The total maximal G_{MI} grew at a faster rate in the presence of $G_{MI\text{-}CCAP}$. Hence, similar to what was observed for current amplitudes, when GPR was stimulated in the absence of CCAP and the total maximal conductance (G_{MI}) was assayed 10 s after the start of retraction, there was insufficient G_{MI} for LG to reach burst threshold (Fig. 3*A*). In contrast, with $G_{MI\text{-}CCAP}$ present, the LG burst was initiated after 10 s of retraction (Fig. 3*B*). Thus, the presence of the additional, GPR-independent $G_{MI\text{-}CCAP}$ reduced the time needed for G_{MI} to bring LG to burst threshold, and thereby reduced the effectiveness of GPR relative to when CCAP was absent (Fig. 3*C*). This result predicted, therefore, that in the biological preparation CCAP would reduce the ability of GPR to prolong the gastric mill retractor phase.

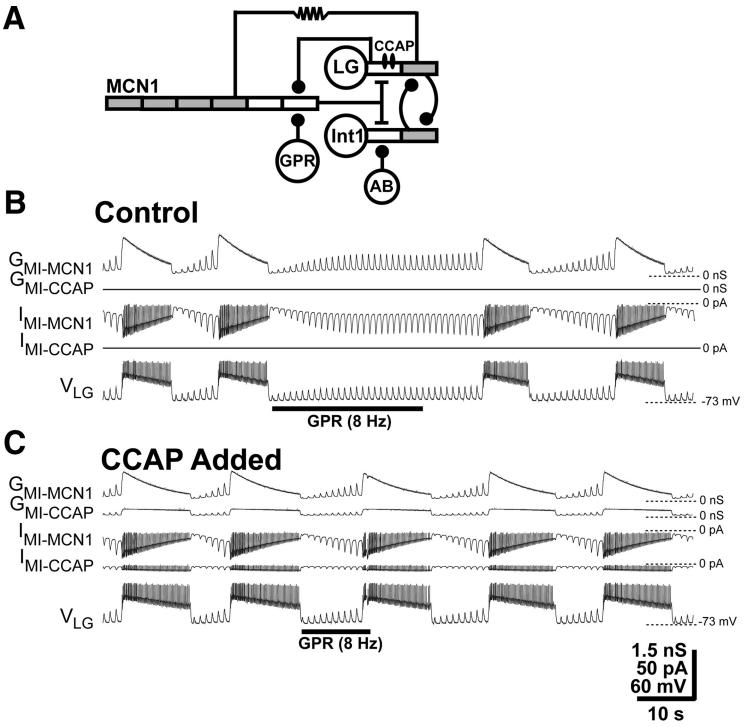

Figure 2. GPR regulation of the gastric mill retractor phase is weakened by the presence of CCAP in a computational model. ***A***, Circuit schematic representing the CCAP modulation of the GPR influence on the MCN1-activated gastric mill CPG in a compartmental model. The model is modified from the original model in the study by Nadim et al. (1998) by the addition of the indicated CCAP and GPR actions. Specifically, as in previously published models, the CCAP-activated conductance was added to the LG neurite compartment (DeLong et al., 2009a), and an inhibitory GPR synapse was added onto the passive terminal compartment of MCN1 (Beenhakker et al., 2005; DeLong et al., 2009b). The gray compartments have active properties mediated by voltage-dependent, Hodgkin–Huxley-like conductances to facilitate action potential generation, whereas the white compartments are passive. Symbols: Filled circles, Synaptic inhibition; T-bars, synaptic excitation; resistor, electrical coupling. ***B***, GPR selectively prolongs the retractor phase of the MCN1-elicited gastric mill rhythm in the absence of CCAP. Under these conditions, the MCN1-elicited inward current ($I_{MI\text{-}MCN1}$) and associated conductance ($G_{MI\text{-}MCN1}$) in LG grew in amplitude during each retractor (LG-silent) phase because of continual MCN1 release of CabTRP Ia, and decayed during each protractor (LG-active) phase because of the LG presynaptic inhibition of $MCN1_{STG}$ (Figs. 1*C*, 2*A*). As in the biological preparation, GPR stimulation during the retractor phase selectively prolonged that phase (Beenhakker et al., 2005; DeLong et al., 2009b). The fast transient events in $I_{MI\text{-}MCN1}$ resulted from the rapid changes in driving force as the LG membrane potential repeatedly approached the I_{MI} reversal potential during the LG action potentials (DeLong et al., 2009a). ***C***, Adding the CCAP-activated conductance ($G_{MI\text{-}CCAP}$) to LG reduced the ability of GPR to prolong retraction. In the presence of $G_{MI\text{-}CCAP}$, the GPR stimulation was less effective in prolonging the retractor phase relative to the control condition (***B***). Note also that, as reported previously (Kirby and Nusbaum, 2007; DeLong et al., 2009b), $G_{MI\text{-}MCN1}$ and $G_{MI\text{-}CCAP}$ exhibited different trajectories during the LG burst, and it is the sustained $G_{MI\text{-}CCAP}$ amplitude during protraction (LG burst) that prolonged this phase relative to the control condition (***B***). As above, the fast transient events in $I_{MI\text{-}MCN1}$ and $I_{MI\text{-}CCAP}$ resulted from the rapid changes in driving force during the LG action potentials.

CCAP modulates the GPR influence on the biological gastric mill rhythm

To test the prediction of the above modeling study, we determined whether CCAP superfusion in the biological preparation comparably modulated the GPR actions on the MCN1-elicited gastric mill rhythm. Specifically, we compared the gastric mill rhythm response to GPR stimulation in control (saline) conditions and in the presence of superfused CCAP.

As proof of principle, we superfused a relatively high CCAP concentration (10^{-7} M) and found that it consistently eliminated the GPR prolongation of the gastric mill retractor phase (Fig. 4*A*). In normal saline, GPR stimulation (5 Hz) during each retractor phase prolonged retraction (pre-GPR, 7.1 ± 1.0 s; during GPR, 20.6 ± 3.5 s; $n = 8$; RM-ANOVA, SNK *post hoc* test, $p < 0.001$) without altering the protractor phase duration (pre-GPR, 5.1 ± 0.8 s; during GPR, 5.2 ± 0.4 s; $n = 8$; RM-ANOVA, SNK *post hoc* test, $p = 0.71$) (Fig. 4*A*). In contrast, during CCAP superfusion, the same GPR stimulation protocol did not alter either retraction duration (pre-GPR, 7.1 ± 1.3 s; during GPR, 9.9 ± 1.7 s; $n = 8$; RM-ANOVA, SNK *post hoc* test, $p = 0.32$) or protraction duration (pre-GPR, 7.1 ± 0.6 s; during GPR, 7.0 ± 0.8 s; $n = 8$; RM-ANOVA, SNK *post hoc* test, $p = 0.97$) (Fig. 4*A*). This CCAP effect resulted from its influence on the GPR action and not on the control duration of the retractor phase, because the retraction duration was the same in saline and CCAP in the absence of GPR stimulation (RM-ANOVA, SNK *post hoc* test, $p = 0.99$) (DeLong et al., 2009a), whereas the retraction duration during GPR stimulation was longer in saline than in the presence of CCAP (RM-ANOVA, SNK *post hoc* test, $p < 0.001$).

Superfusing a lower CCAP concentration (10^{-9} M), within the concentration range likely present in the hemolymph *in vivo* (Phlippen et al., 2000) [Kirby and Nusbaum (2007), their Discussion], also weakened the GPR action on the MCN1–gastric mill rhythm. As occurred in the above experiments using the higher CCAP concentration, in these preparations GPR stimulation prolonged retraction during saline superfusion (pre-GPR, 8.9 ± 1.3 s; during GPR, 20.1 ± 4.4 s; $n = 4$; $p < 0.05$) but not during CCAP (10^{-9} M) application (pre-GPR, 8.3 ± 1.3 s; during GPR, 9.8 ± 1.6 s; $n = 4$; $p = 0.25$). Also as above, protraction duration was not altered by GPR stimulation either during saline ($n = 4$; $p = 0.19$) or CCAP (10^{-9} M) superfusion ($n = 4$; $p = 0.22$). These results were consistent with our model prediction that CCAP gates out the GPR effect on the MCN1-elicited gastric mill rhythm.

To further assess the effectiveness of this CCAP gating effect, we tested whether CCAP also suppressed the gastric mill rhythm response to stimulating GPR at a faster frequency (10–15 Hz). Specifically, under control conditions, faster frequency stimulation of GPR consistently maintained the retractor phase until after the stimulation was terminated (30 s: $n = 3$ of 3) (Fig. 4*B*).

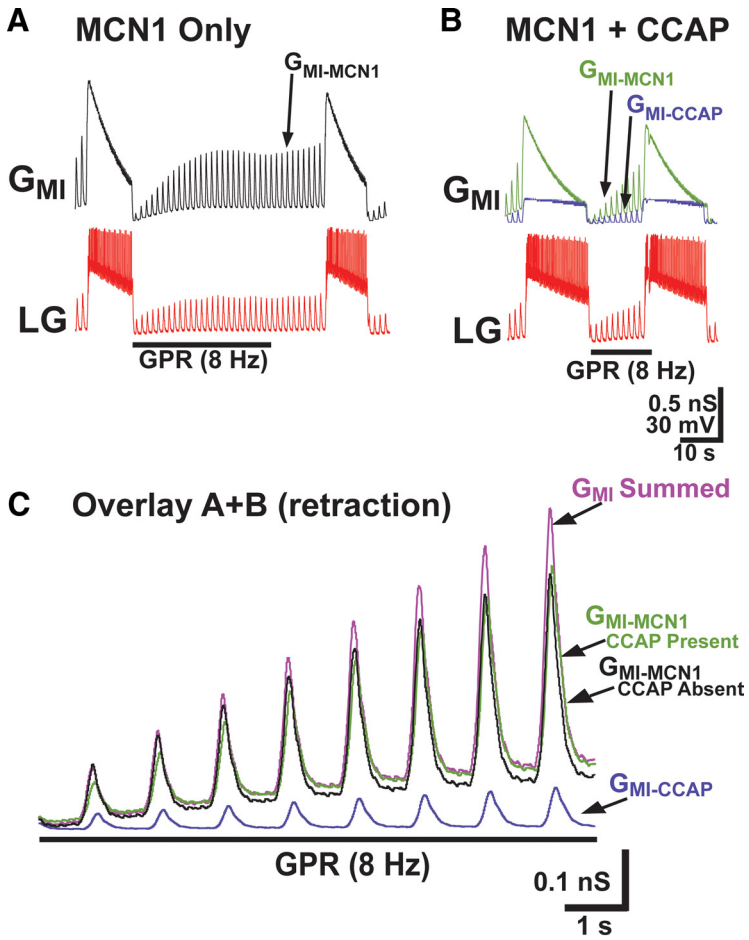

Figure 3. The altered G_{MI} dynamics in the LG neuron when CCAP is present reduces the ability of GPR to prolong the gastric mill retractor phase in a computational model. ***A***, During the control gastric mill rhythm, G_{MI} in LG was entirely attributable to input from MCN1 ($G_{MI-MCN1}$). Under these conditions, GPR stimulation prolonged the retractor phase (Fig. 2*B*) by reducing the rate of buildup of $G_{MI-MCN1}$ caused by the GPR presynaptic inhibition of MCN1 (Beenhakker et al., 2005; DeLong et al., 2009b). ***B***, When CCAP was present, G_{MI} consisted of the summed components contributed by MCN1 ($G_{MI-MCN1}$) and CCAP ($G_{MI-CCAP}$). Under this condition, GPR stimulation was less effective in prolonging retraction. ***C***, The presence of CCAP during MCN1 stimulation produced a summed maximal G_{MI} in LG during the retractor phase that grew at a faster rate than when G_{MI} was entirely contributed by MCN1. The G_{MI} traces from ***A*** and ***B*** are overlaid, for durations that span the first ~10 s of the retractor phase during GPR stimulation. With CCAP both present and absent, the amplitude of $G_{MI-MCN1}$ grew steadily during retraction. However, the presence of CCAP produced a summed maximal G_{MI} that grew at a faster rate, because it was additionally composed of the GPR-independent $G_{MI-CCAP}$. Hence, the summed maximal G_{MI} in the presence of CCAP enabled LG to attain burst threshold sooner.

In contrast, in the presence of CCAP (10^{-7} M) the LG neuron consistently escaped during the faster frequency GPR stimulation and fired a burst before the 30 s of GPR stimulation was completed ($n = 3$ of 3) (Fig. 4*B*), as also occurred during 5 Hz GPR stimulation (Fig. 4*A*). Overall, CCAP gated out the influence of this faster frequency GPR stimulation ($n = 3$; RM-ANOVA, SNK *post hoc* test, GPR vs pre-GPR in CCAP, $p = 0.13$), as it had done to the 5 Hz GPR stimulation, although in two of these three preparations the effect of the faster frequency GPR stimulation was reduced but not eliminated (Fig. 4*B*). As was the case for the 5 Hz stimulation studies reported above (Fig. 4*A*), this CCAP action resulted from its ability to limit the retractor phase duration during the faster frequency GPR stimulation relative to the duration that occurred during saline superfusion (RM-ANOVA, SNK *post hoc* test, $p < 0.005$). This conclusion was supported by the fact that CCAP did not alter retraction duration relative to saline superfusion in the absence of GPR stimulation (RM-ANOVA, SNK *post hoc* test, $p = 0.90$) (Fig. 4*B*).

The above experiments were performed by stimulating GPR during the retractor phase to mimic the likely GPR *in vivo* activity pattern (Katz et al., 1989). However, because CCAP reduced the ability of GPR to prolong retraction, the total time during which GPR was stimulated was less in CCAP than in control conditions. To eliminate this potential confound, we also tested the ability of a fixed duration (30 s) tonic GPR stimulation (5 Hz) to regulate the gastric mill rhythm under both conditions. Extending GPR stimulation through the protractor phase did not introduce an additional complication because stimulating GPR during protraction does not alter LG activity or either phase of the rhythm (DeLong et al., 2009b).

Using tonic GPR stimulation in normal saline, GPR again prolonged the retractor phase (pre-GPR, 8.1 ± 1.5 s; during GPR, 22.4 ± 6.7 s; $n = 4$; $p < 0.05$) without altering the protractor phase duration (pre-GPR, 3.8 ± 0.9 s; during GPR, 3.4 ± 0.6 s; $n = 4$; $p = 0.38$). In contrast, the same tonic GPR stimulation in the presence of CCAP (10^{-7} M) did not alter the duration of either the retractor phase (pre-GPR, 10.5 ± 2.6 s; during GPR, 13.0 ± 3.6 s; $n = 4$; $p = 0.30$) or the protractor phase (pre-GPR, 6.3 ± 0.7 s; during GPR, 6.4 ± 1.1 s; $n = 4$; $p = 0.47$). These results confirmed our model prediction that CCAP gates out the GPR regulation of the gastric mill rhythm.

CCAP gates out the GPR regulation of the gastric mill rhythm by activating $G_{MI-CCAP}$ in LG

CCAP excites several gastric mill neurons in addition to LG, including the gastric mill CPG neuron Int1 (Kirby and Nusbaum, 2007). Therefore, although our model predicted that the induction of $G_{MI-CCAP}$ in LG was sufficient for gating out the GPR effect on the gastric mill rhythm, it remained possible that one or more other CCAP actions contributed to this effect. Consequently, we tested the hypothesis that the CCAP-activated G_{MI} in LG was necessary and sufficient for gating out this GPR effect. To this end, we first tested the ability of a simulated version of $G_{MI-CCAP}$, injected into LG using the dynamic clamp, to mimic the bath-applied CCAP regulation of the GPR action on the gastric mill rhythm. For these experiments, we used the same dynamic-clamp conductance used previously, which was based on voltage-clamp recordings of $I_{MI-CCAP}$ in LG (DeLong et al., 2009a).

We compared the effect of GPR stimulation (5 Hz) under control conditions to the same stimulation during dynamic-clamp injection of $G_{MI-CCAP}$ in LG. As above, under control conditions the GPR stimulation prolonged retraction (pre-GPR, 6.3 ± 1.0 s; during GPR, 18.6 ± 4.7 s; $n = 5$; $p < 0.05$) without altering protraction duration (pre-GPR, 5.8 ± 1.3 s; during GPR, 6.7 ± 1.2 s; $n = 5$; $p = 0.30$) (Fig. 5*A*). When the same stimulation was performed while dynamic-clamp $I_{MI-CCAP}$ ($G_{MI-CCAP}$, 20 nS) was injected into LG, there was no change in the duration of either retraction (pre-GPR, 7.9 ± 1.5 s; during GPR, 11.0 ± 2.6 s; $n = 5$;

$p = 0.17$) or protraction (pre-GPR, 8.6 ± 2.2 s; during GPR, 9.0 ± 2.0 s; $n = 5$; $p = 0.45$) (Fig. 5*B*). These results supported the hypothesis that the presence of $G_{MI\text{-}CCAP}$ in LG was sufficient to gate out the GPR influence on the MCN1-elicited gastric mill rhythm.

To test the necessity of $G_{MI\text{-}CCAP}$ in LG in the aforementioned gating action, we used the dynamic clamp to inject $I_{MI\text{-}CCAP}$ into LG using a negative conductance version of $G_{MI\text{-}CCAP}$ (−20 or −30 nS) during CCAP (10^{-7} M) bath application. This approach is sufficient to eliminate the ability of CCAP to prolong the gastric mill protractor phase (DeLong et al., 2009a). In the absence of this dynamic-clamp manipulation, GPR stimulation (5–6 Hz) prolonged retraction ($n = 5$; $p < 0.005$) without altering protraction duration ($n = 5$; $p = 0.35$) during saline superfusion but did not alter the duration of either phase (retraction: $n = 5$, $p = 0.16$; protraction: $n = 5$, $p = 0.43$) during CCAP superfusion (Fig. 6*A*,*B*). In contrast, when $I_{MI\text{-}CCAP}$ was injected into LG using the negative version of $G_{MI\text{-}CCAP}$ during CCAP superfusion, GPR stimulation prolonged retraction (pre-GPR, 10.4 ± 1.9 s; during GPR, 22.5 ± 1.0 s; $n = 5$; $p < 0.005$), without altering protraction duration (pre-GPR, 4.7 ± 0.8 s; during GPR, 4.0 ± 0.7 s; $n = 5$; $p = 0.26$) (Fig. 6*C*). The duration of the prolonged retractor phase under these conditions was the same as occurred during saline superfusion (RM-ANOVA, SNK *post hoc* test, $p = 0.90$). The ability of these dynamic-clamp injections with the negative $G_{MI\text{-}CCAP}$ to eliminate the CCAP gating action on the GPR regulation of the gastric mill rhythm supported the hypothesis that the presence of $G_{MI\text{-}CCAP}$ in LG was necessary and sufficient to mediate this CCAP effect.

Discussion

We have shown that the presence of a peptide hormone weakens or suppresses proprioceptor feedback to a motor circuit. This action occurs despite the fact that the hormone neither influences the proprioceptor synaptic action nor directly alters motor activity during the phase of the motor pattern that is influenced by this proprioceptor. Previous work showed that, during saline superfusion, the proprioceptor neuron GPR selectively prolongs the gastric mill retractor phase by reducing MCN1 release of the peptide transmitter CabTRP Ia (Fig. 7*A*,*B*) (Beenhakker et al., 2005; DeLong et al., 2009b). This action reduces the activation rate of the modulator (MCN1-released CabTRP Ia)-

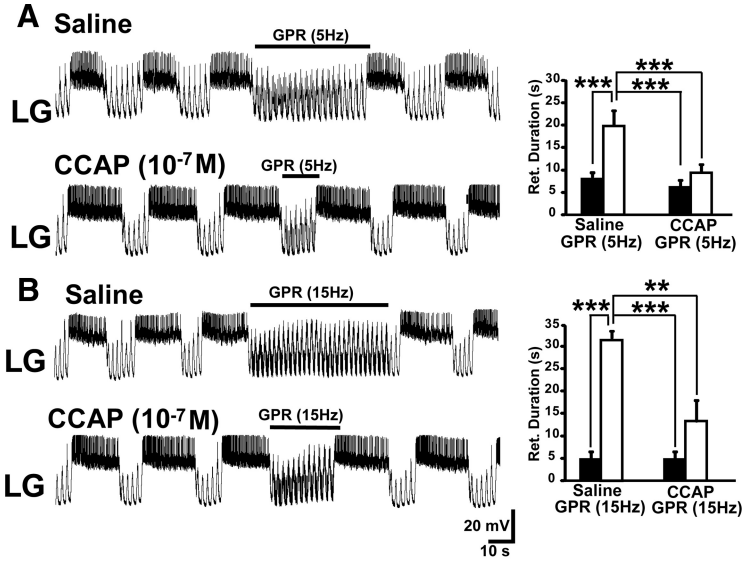

Figure 4. CCAP superfusion reduces the effectiveness of GPR stimulation during the biological MCN1-elicited gastric mill rhythm. ***A***, Left, GPR stimulation selectively prolonged retraction (LG silent) under control (saline) conditions during the MCN1–gastric mill rhythm. In contrast, the same level of GPR stimulation barely prolonged retraction during CCAP superfusion. Note that, as usual, CCAP prolonged LG burst duration (Kirby and Nusbaum, 2007). Most hyperpolarized V_m: saline, −62 mV; CCAP, −65 mV. Right, Cumulative data showing that GPR consistently prolonged the gastric mill retractor phase during saline superfusion. In contrast, GPR stimulation did not alter retraction duration in the presence of CCAP (10^{-7} M; RM-ANOVA, SNK *post hoc* test, $p = 0.32$; $n = 5$). The retraction duration was also prolonged by the GPR stimulation in saline compared with the same stimulation in the presence of CCAP, whereas there was no difference in the duration of this phase during the two control conditions (RM-ANOVA, SNK *post hoc* test, $p = 0.99$; $n = 5$). ***RM-ANOVA, SNK *post hoc* text, $p < 0.001$; $n = 5$. The black bars represent gastric mill cycles without GPR stimulation, and the white bars represent cycles with GPR stimulation. Error bars indicate SEM. ***B***, Left, Increasing the GPR stimulation frequency prolonged the gastric mill retractor phase in saline but still failed to maintain its effectiveness during CCAP superfusion. Most hyperpolarized V_m: Both panels, −58 mV. Right, Cumulative data showing that increasing the GPR stimulation frequency prolongs the retractor phase during saline superfusion. However, this faster stimulation frequency was not sufficient to overcome the influence of CCAP (10^{-7} M) (RM-ANOVA, SNK *post hoc* test, $p = 0.13$; $n = 3$). Also, as in ***A***, the retraction duration was distinct during the GPR stimulations in saline and CCAP, but not during the two control conditions (RM-ANOVA, SNK *post hoc* test, $p = 0.90$; $n = 3$). ***RM-ANOVA, SNK *post hoc* test, $p < 0.001$; **RM-ANOVA, SNK *post hoc* test, $p < 0.005$. The bars are as above.

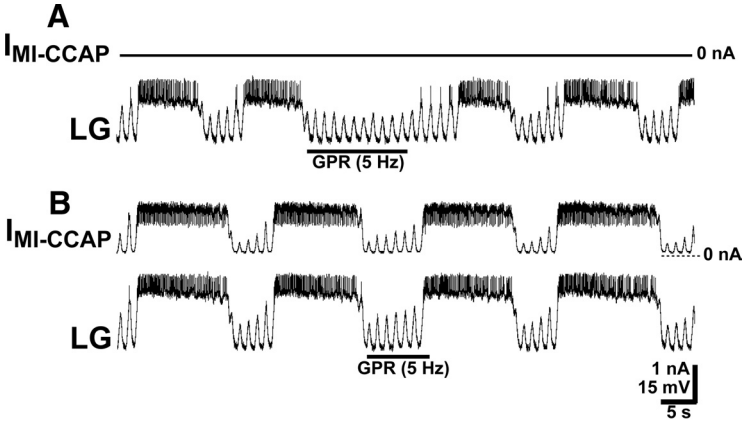

Figure 5. Dynamic-clamp injection of the CCAP-activated current ($I_{MI\text{-}CCAP}$) into LG is sufficient to mimic the ability of bath-applied CCAP to weaken the GPR action on the MCN1-elicited gastric mill rhythm. ***A***, In the absence of the dynamic-clamp injection ($I_{MI\text{-}CCAP}$, 0 nA), GPR stimulation during the gastric mill retractor phase selectively prolonged that phase. Most hyperpolarized V_m, −59 mV. ***B***, Dynamic clamp depolarizing current injection of $I_{MI\text{-}CCAP}$ into LG mimicked the ability of bath-applied CCAP to weaken GPR regulation of the gastric mill rhythm. While $I_{MI\text{-}CCAP}$ was being injected into LG, GPR stimulation barely prolonged the retractor phase. The fast transient, downward deflections in $I_{MI\text{-}CCAP}$ during the LG action potentials resulted from the reduced driving force as the LG membrane potential approached the I_{MI} reversal potential. Note the increased LG burst duration during $I_{MI\text{-}CCAP}$ injection, as also occurs during CCAP bath application (Kirby and Nusbaum, 2007). Most hyperpolarized V_m, −60 mV. Both panels are from the same LG recording.

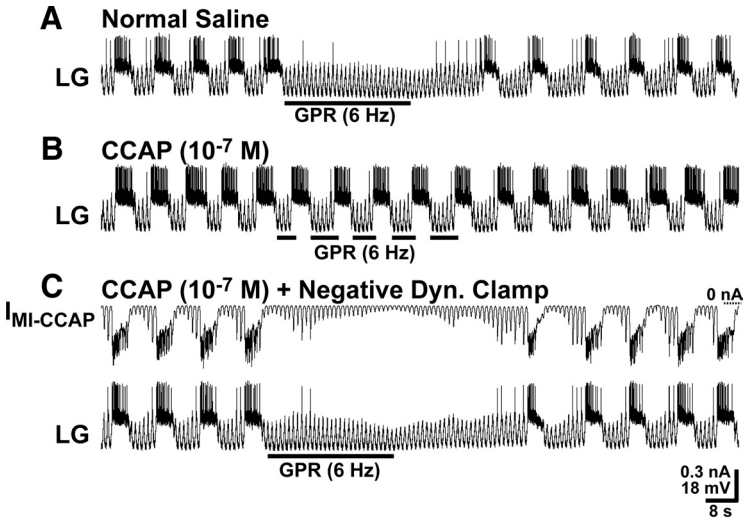

Figure 6. The presence of $G_{MI\text{-}CCAP}$ in LG is necessary for CCAP to gate out the GPR prolongation of the gastric mill retractor phase. ***A***, During saline superfusion, GPR stimulation selectively prolonged the retractor phase of the MCN1– gastric mill rhythm. Most hyperpolarized V_m, −71 mV. ***B***, During CCAP superfusion, the effect of GPR stimulation on the retractor phase was gated out, despite stimulating GPR during successive retractor phases. Most hyperpolarized V_m, −74 mV. ***C***, During CCAP superfusion, dynamic-clamp-mediated injection $I_{MI\text{-}CCAP}$ into LG using a negative version of $G_{MI\text{-}CCAP}$ (note hyperpolarizing current injections) eliminated the ability of CCAP to gate out the GPR action on the MCN1– gastric mill rhythm. Most hyperpolarized V_m, −74 mV. All three panels are from the same LG recording.

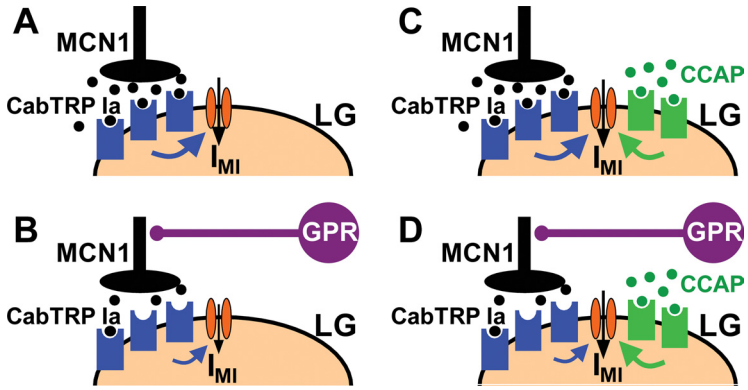

Figure 7. Summary schematic of the mechanism by which CCAP gates out the GPR regulation of the MCN1– gastric mill rhythm. ***A***, During the normal gastric mill rhythm retractor phase, with no CCAP present, MCN1 released CabTRP Ia (filled black circles) binds to receptors on LG (blue geometric shapes) to activate I_{MI} via an unidentified metabotropic pathway (blue arrow). The downward pointing arrow depicts activated I_{MI}. ***B***, During the gastric mill retractor phase with GPR stimulation and no CCAP present, CabTRP Ia release from MCN1 is reduced, resulting in a reduced rate of activation of I_{MI} (note smaller size of metabotropic- and I_{MI}-associated arrows). ***C***, During the gastric mill retractor phase with CCAP present (filled green circles), I_{MI} in LG is coactivated by MCN1-released CabTRP Ia and bath-applied CCAP. ***D***, During the gastric mill retractor phase with GPR stimulation and CCAP present, GPR still reduces CabTRP Ia release from MCN1. However, because $I_{MI\text{-}CCAP}$ in LG is not regulated by GPR activity and can compensate for the reduced amount of $I_{MI\text{-}MCN1}$, the GPR effect on I_{MI}, and hence on the gastric mill retractor phase, is reduced.

activated inward conductance $G_{MI\text{-}MCN1}$ in the gastric mill CPG neuron LG during retraction, thereby slowing the LG neuron escape from Int1-mediated inhibition (Figs. 2, 3). Furthermore, the peptide hormone CCAP selectively prolongs the gastric mill protractor phase via a parallel activation, with MCN1, of G_{MI} in the LG neuron (Fig. 7*C*) (Kirby and Nusbaum, 2007; DeLong et al., 2009a).

Our computational model predicted that CCAP gates out the GPR effect on the MCN1-elicited gastric mill rhythm. The model further predicted that this gating effect results specifically from CCAP providing a parallel pathway for activating G_{MI} in the LG neuron that is MCN1 independent, and therefore not regulated by GPR (Fig. 7*D*). We verified these model predictions by demonstrating that either bath-applied CCAP or dynamic-clamp injection of $G_{MI\text{-}CCAP}$ into the biological LG neuron gated out the GPR action on the gastric mill rhythm, whereas dynamic-clamp injections using a negative version of $G_{MI\text{-}CCAP}$ into LG during CCAP bath application restored this GPR action. Thus, CCAP-activated G_{MI} in LG is necessary and sufficient to gate out the sensory feedback action of GPR on the MCN1-elicited gastric mill rhythm.

Modulation of sensory input to a motor circuit

Sensory neuron activity is often regulated at the level of the sensory transduction apparatus and/or near their axon terminals in the CNS (Birmingham et al., 2003; Bewick et al., 2005; Kohlmeier et al., 2006; Rossignol et al., 2006; Blitz and Nusbaum, 2007; Kindt et al., 2007; Barrière et al., 2008; Glanzman, 2008; Maher and Westbrook, 2008; Pírez and Wachowiak, 2008; Petzold et al., 2009; Rudomin, 2009). Regulation of sensory input also occurs via actions in the postsynaptic targets of the sensory neurons (Edwards et al., 2002; Le Bon-Jego et al., 2006; Glanzman, 2008). Here, we demonstrated that modulation of sensory transmission can also result from a downstream circuit action. Specifically, the peptide hormone CCAP gates out the effect of the proprioceptor neuron GPR, not by a direct action on GPR or on the postsynaptic target (MCN1) by which GPR alters gastric mill CPG output, but by activating a voltage-dependent ionic current in a circuit neuron downstream of the relevant GPR target. This downstream action enables the circuit to compensate for the sensory-mediated reduction in modulatory drive. Insofar as CCAP, as a circulating hormone, also has access to the associated peripheral structures, it remains possible that CCAP additionally modulates this sensorimotor pathway by influencing GPR sensitivity to muscle stretch or spike initiation, as do several other neuromodulators (Birmingham et al., 2003).

Modulating neuronal properties to alter motor circuit sensitivity to sensory feedback

CCAP alters the modulatory state of the gastric mill CPG without substantially changing the gastric mill motor pattern, even when applied at relatively high concentrations (Kirby and Nusbaum, 2007). This limited influence on motor circuit output results from its convergent activation, with MCN1-released CabTRP Ia, of G_{MI} in LG (DeLong et al., 2009a). In contrast, modulation frequently causes considerable change in motor circuit output (Marder et al., 2005; Dickinson, 2006; Marder and Bucher, 2007; Doi and Ramirez, 2008). Despite its modest direct effect on the

gastric mill rhythm, the same CCAP concentrations enable a substantially altered responsiveness to a sensory input.

Our previous work (DeLong et al., 2009a) established that, despite its modest effect on the gastric mill rhythm, CCAP changes the G_{MI} dynamics in the LG neuron. These altered dynamics not only cause a modest prolongation of protraction but enable the retractor phase to retain its control duration. Both of these effects result from $G_{MI\text{-}CCAP}$ reducing the impact of synaptic regulation of G_{MI} by LG inhibition of $MCN1_{STG}$ (DeLong et al., 2009a). The same altered dynamics underlie the ability of CCAP to gate out the GPR inhibition of $MCN1_{STG}$ during retraction (this study).

The modulatory state of a network can also be altered without affecting network output when a modulator that normally changes network output is present at a low concentration. For example, in the lobster STNS such a latent state change enables the cardiac sac network to become responsive to an otherwise ineffective, second peptide modulator (Dickinson et al., 1997). The mechanism underlying this latter effect has yet to be elucidated, although based on more recent work on crab pyloric circuit neurons, it may be mediated by a convergent activation of G_{MI} (Swensen and Marder, 2000, 2001). A peptide modulator also strengthens considerably the sole (inhibitory) pyloric circuit feedback synapse to the pyloric pacemaker neurons, yet this enhanced synaptic action does not alter the pyloric cycle frequency (Thirumalai et al., 2006). As is the case for the relative impact of CCAP on the gastric mill rhythm and GPR regulation of this rhythm, this strengthened synaptic feedback may well influence pyloric cycle frequency in the presence of a parallel perturbation.

There is a growing literature demonstrating the ability of neurons to express broadly different levels of intrinsic and synaptic conductances and yet produce conserved neuronal and circuit activity patterns (Cymbalyuk et al., 2002; Prinz et al., 2004; Marder and Goaillard, 2006; Saideman et al., 2007b; Goaillard et al., 2009; Grashow et al., 2009; Olypher and Calabrese, 2009; Taylor et al., 2009). These studies suggest that, although the system output remains stable in the presence of varying intrinsic and synaptic conductances, the differing combinations of conductance levels can make a neuron or network differentially sensitive to a particular perturbation. Our study establishes, in a biological system, that conserved motor output can indeed mask a differential sensitivity to an identified synaptic input. Specifically, although the versions of the gastric mill rhythm in the presence and absence of CCAP are similar, the distinct G_{MI} dynamics in LG during these two rhythms enable the gastric mill circuit to respond differently to input from GPR. By extension, this study also highlights the care that should be taken when inferring conserved neuronal and circuit properties on the basis of an unchanging or modest change in motor output in the presence of a modulatory action.

We did not explore the full range of CCAP concentrations that influence the GPR action on the gastric mill rhythm. However, the fact that 10^{-9} M CCAP was a consistently effective concentration for gating out this GPR feedback effect suggests that this hormonal action is likely to occur in the intact animal. The steady-state and feeding-related concentration of CCAP circulating in the *C. borealis* hemolymph is not known, but studies in other crabs (*Carcinus maenas*, *Orconectes limosus*) suggest that 10^{-9} M CCAP is within the normal range of concentrations that occur during periods of enhanced release (Phlippen et al., 2000) [Kirby and Nusbaum (2007), their Discussion]. One condition in which elevated CCAP levels appear to occur is in food-deprived *C. borealis* (Chen et al., 2009). The *in vivo* conditions, however, may have additional degrees of freedom because of both the presence of other circulating hormones and the fact that, although GPR likely provides feedback whenever the gastric mill rhythm is occurring, its firing rate and pattern can be modulated at/near its sensory transduction site (Katz et al., 1989; Birmingham et al., 1999, 2003).

In conclusion, there is growing support for the ability of neuronal ensembles to use a variety of cellular and synaptic properties to generate a relatively stable circuit output. These studies have raised the issue of whether these apparently unchanging outputs maintain a consistent response to perturbations. The present study establishes that this is not necessarily the case, as the regulation of the MCN1-elicited gastric mill rhythm by the proprioceptor neuron GPR is weakened by the presence of the peptide hormone CCAP, despite the fact that CCAP causes only a modest change in one phase of this rhythm and does not change the phase of the rhythm affected by the input from GPR.

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