Behavioral/Systems/Cognitive

Mechanosensory Gating of Proprioceptor Input to Modulatory Projection Neurons

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Sensorimotor gating commonly occurs at sensory neuron synapses onto motor circuit neurons and motor neurons. Here, using the crab stomatogastric nervous system, we show that sensorimotor gating also occurs at the level of the projection neurons that activate motor circuits. We compared the influence of the gastro-pyloric receptor (GPR) muscle stretch-sensitive neuron on two projection neurons, modulatory commissural neuron 1 (MCN1) and commissural projection neuron 2 (CPN2), with and without a preceding activation of the mechanosensory ventral cardiac neurons (VCNs). MCN1 and CPN2 project from the paired commissural ganglia (CoGs) to the stomatogastric ganglion (STG), where they activate the gastric mill (chewing) motor circuit. When stimulated separately, the GPR and VCN neurons each elicit the gastric mill rhythm by coactivating MCN1 and CPN2. When GPR is instead stimulated during the VCN-gastric mill rhythm, it slows this rhythm. This effect results from a second GPR synapse onto MCN1 that presynaptically inhibits its STG terminals. Here, we show that, during the VCN-triggered rhythm, the GPR excitation of MCN1 and CPN2 in the CoGs is gated out, leaving only its influence in the STG. This gating effect appears to occur within the CoG and does not result from a ceiling effect on projection neuron firing frequency. Additionally, this gating action enables GPR to either activate rhythmic motor activity or act as a phasic sensorimotor feedback system. These results also indicate that the site of sensorimotor gating can occur at the level of the projection neurons that activate a motor circuit.

Key words: **central pattern generator; stomatogastric; gastric mill circuit; state dependent; rhythms; convergence**

Introduction

Neuronal systems regulate incoming signals in a state-dependent manner. One well-documented mechanism for gating sensory inputs to motor systems is the presynaptic inhibition of sensory synapses onto central pattern generating (CPG) circuits and their associated motor neurons (Nusbaum et al., 1997; Cattaert et al., 2002; Rudomin, 2002; Frost et al., 2003; Katz, 2003; Rossignol et al., 2006; Blitz and Nusbaum, 2007). This presynaptic inhibition originates from descending influences, the motor circuit itself, or other sensory neurons. Sensory inputs also influence descending projections to CPGs, thereby initiating or terminating entire motor programs (Viana di Prisco et al., 2000; Perrins et al., 2002; Beenhakker and Nusbaum, 2004; Blitz et al., 2004). Little is known, however, regarding the extent to which this latter site is regulated in a state-dependent manner.

We are assessing the presence and function of state-dependent sensory input to projection neurons that influence the gastric mill (chewing) CPG in the stomatogastric ganglion (STG) of the crab *Cancer borealis* (Nusbaum and Beenhakker, 2002; Marder and Bucher, 2007). The gastric mill rhythm is activated by several sensory systems, each of which does so via their excitation of projection neurons located primarily in the paired commissural ganglia (CoGs) (Meyrand et al., 1994; Combes et al., 1999; Beenhakker and Nusbaum, 2004; Blitz et al., 2004). One such system in *C. borealis* is a population of mechanosensory neurons, ventral cardiac neurons (VCNs), embedded in the wall of the stomach (Beenhakker et al., 2004). Relatively brief (<2 min) VCN stimulation triggers a long-lasting (>10 min) gastric mill rhythm via their activation of two projection neurons, modulatory commissural neuron 1 (MCN1) and commissural projection neuron 2 (CPN2), which are present as single copies in each CoG (Beenhakker and Nusbaum, 2004).

The crab gastric mill rhythm is also influenced by a bilaterally symmetric pair of muscle-stretch receptor neurons called the gastro-pyloric receptor neurons (GPRs) (Katz and Harris-Warrick, 1989; Katz et al., 1989). In the isolated stomatogastric nervous system (STNS), rhythmic GPR stimulation elicits the gastric mill rhythm, also via activation of MCN1 and CPN2 (Blitz et al., 2004). Despite activating the gastric mill rhythm by targeting the same projection neurons, the GPR-elicited rhythm is slower and shorter-lasting than the one triggered by VCN stimulation (Blitz et al., 2004). Stimulating GPR during the VCN-triggered gastric mill rhythm slows this rhythm as well by selectively prolonging the gastric mill retractor phase, at least partly via GPR inhibition of the STG terminals of MCN1 ($MCN1_{STG}$) (Beenhakker et al., 2005).

In this paper, we examined the GPR influence on MCN1 and

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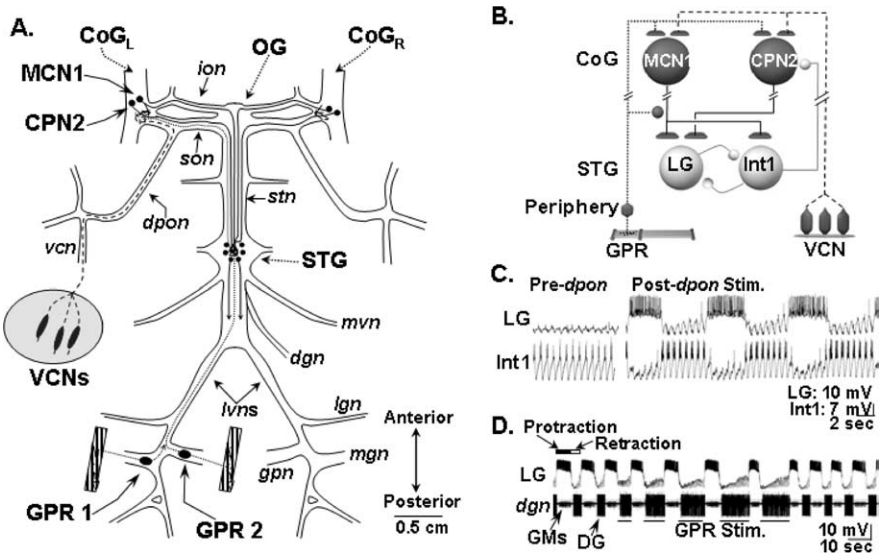

Figure 1. ***A***, Schematic of the stomatogastric nervous system, including somata location and axonal pathways of the GPR and VCN sensory neurons and the projection neurons MCN1 and CPN2. Each GPR neuron projects to and arborizes within the STG and each CoG. There is a single MCN1 and CPN2 in each CoG. MCN1 projects through the inferior oesophageal nerve (*ion*) and *stn* to innervate the STG, whereas CPN2 projects through the superior oesophageal nerve (*son*) and *stn* to innervate the STG. *dgn*, Dorsal gastric nerve; *lgn*, lateral gastric nerve; *lvn*, lateral ventricular nerve; *mgn*, medial gastric nerve; *mvn*, medial ventricular nerve. ***B***, Proprioceptor (GPR) and mechanoreceptor (VCN) neuron actions on projection neurons (MCN1, CPN2) that activate the gastric mill circuit (e.g., LG, Int1) (Beenhakker and Nusbaum, 2004; Beenhakker et al., 2004; Blitz et al., 2004). T-bars, Synaptic excitation; circles, synaptic inhibition. Line breaks in the sensory and projection neuron axons represent additional distance between the STG and CoG. ***C***, Simultaneous intracellular recordings of the LG protractor and Int1 retractor neurons before and during the VCN-triggered gastric mill rhythm. Most hyperpolarized Vm: LG, −70 mV; Int1, −52 mV. ***D***, Stimulating GPR at the behaviorally appropriate time slows the VCN-elicited gastric mill rhythm by prolonging the retractor phase. Rhythmic stimulation of GPR (bars, 5 Hz) during the retractor phase (DG neuron active) of a VCN-triggered gastric mill rhythm caused a progressively increasing prolongation of the retractor phase (Beenhakker et al., 2005).

CPN2 in the CoG during the VCN-triggered gastric mill rhythm. We stimulated GPR during each retractor phase of the VCN-triggered rhythm based on its expected activity pattern *in situ* (Katz and Harris-Warrick, 1989, 1991). During the VCN-triggered rhythm, GPR lost its ability to excite MCN1 and CPN2 in the CoG but retained its ability to inhibit $MCN1_{STG}$ and cause the resulting prolongation of the retractor phase. Thus, sensorimotor gating can switch proprioceptor action from motor pattern activation, via excitation of modulatory projection neurons, to phasic regulation of the motor circuit driven by these projection neurons.

Materials and Methods

Experimental preparation. Male Jonah crabs (*C. borealis*) were obtained from the Marine Biological Laboratory (Woods Hole, MA) and from Commercial Lobster and Seafood (Boston, MA). Animals were maintained in chilled (10–12°C), aerated, filtered, and recirculated artificial seawater until experimentation. Before the dissection, each crab was anesthetized by packing in ice for at least 30 min. The foregut was then removed and submerged in cold saline (see below) to dissect the STNS from the foregut. The isolated STNS (see Fig. 1*A*) was then pinned down in a silicone elastomer-lined Petri dish (Sylgard 184; KR Anderson, Santa Clara, CA) filled with cold saline. During experimentation, the STNS was superfused continuously (7–12 ml/min) with cold (10–12°C) saline of the following composition (in mM): 439 NaCl, 26 $MgCl_2$, 13 $CaCl_2$, 11 KCl, 10 Trizma base, and 5 maleic acid, pH 7.4–7.6. In some experiments, transmitter release was eliminated selectively in the STG by superfusing it with saline that contained a reduced (0.1× normal) concentration of Ca^{2+} plus a compensatory addition of Mn^{2+} ("low-Ca^{2+} saline") (Blitz and Nusbaum, 1997). Low-Ca^{2+} saline contained the following (in mM): 439 NaCl, 26 $MgCl_2$, 1.3 $CaCl_2$, 11.7 $MnCl_2$, 11 KCl, 10 Trizma base, and 5 maleic acid, pH 7.4–7.6. At these times, a Vaseline (Lab Safety Supply, Janesville, WI) wall that crossed the midpoint of the stomatogastric nerve (*stn*) separated the STG from the CoGs and oesophageal ganglion (OG), enabling us to superfuse separately the two halves of the system.

Electrophysiology. Standard intracellular and extracellular recording techniques were used for this project (Beenhakker and Nusbaum, 2004; Beenhakker et al., 2004). Briefly, intracellular recordings were made by impaling somata with sharp glass microelectrodes (15–30 MΩ) filled with either 0.6 M K_2SO_4 plus 10 mM KCl or 4 M K-acetate plus 20 mM KCl. Intracellular signals were amplified with Axoclamp 2B amplifiers (Molecular Devices, Sunnyvale, CA). All intracellular current injections were performed in the discontinuous current-clamp (DCC) mode (sampling rate, 2–5 KHz). Intracellular recordings were aided by illuminating the desheathed ganglia with light transmitted through a dark-field condenser (Nikon, Tokyo, Japan).

Extracellular recordings from nerves were obtained by placing one of two stainless steel electrode wires within a petroleum jelly (Vaseline; Lab Safety Supply) well made to electrically isolate a section of the nerve from the bath, and placing the second wire in the bath compartment. Extracellular signals were amplified through two stages (stage 1: model 1700 AC Amplifier, AM Systems, Carlsborg, WA; stage 2: model 410 AC/DC Amplifier, Brownlee Precision, Santa Clara, CA). Extracellular nerve stimulation was achieved by placing the two recording leads into a stimulus isolation unit controlled by an S88 stimulator (Astromed/Grass Instruments, West Warwick, RI).

The VCN neurons were selectively activated by extracellular stimulation of either the ventral cardiac nerve (*vcn*) or dorsal posterior oesophageal nerve (*dpon*) (see Fig. 1*A*) (Beenhakker and Nusbaum, 2004; Beenhakker et al., 2004). The GPR neuron was activated by stimulating the gastropyloric nerve (*gpn*) (see Fig. 1*A*) (Katz and Harris-Warrick, 1991; Blitz et al., 2004). These stimulations are sufficient to enable each of these sensory systems to activate the projection neurons MCN1 and CPN2, and repetitive stimulation of either the VCNs or GPR elicits the gastric mill rhythm through their activation of MCN1 and CPN2 (see Fig. 1*B*,*C*) (Beenhakker and Nusbaum, 2004; Blitz et al., 2004). Neurons were identified by their patterns of activity, interactions with other neurons, and axonal branching patterns (Weimann et al., 1991; Beenhakker and Nusbaum, 2004; Beenhakker et al., 2004).

During the VCN-triggered gastric mill rhythm, GPR was stimulated during a succession of retractor phases, consistent with its activity pattern during the gastric mill rhythm in semi-intact preparations (see Fig. 1*D*) (Katz and Harris-Warrick, 1989). The retractor phase was defined as the duration between the end of one lateral gastric (LG) neuron burst and the onset of the next LG neuron burst.

To determine the effects of GPR stimulation on the VCN-triggered gastric mill rhythm in the partial absence of sensorimotor gating, we increased the MCN1 firing frequency during these GPR stimulations by directly depolarizing MCN1 in the CoG via intrasomatic depolarizing current injection. In these experiments, we first monitored the gastric mill cycle period for 7–10 cycles after VCN stimulation to establish a stable gastric mill cycle period and LG neuron burst duration. After this control period, we simultaneously depolarized MCN1 (<2.0 nA in each experiment) to increase its firing frequency and stimulated GPR (5–10 Hz tonic stimulation) for four to six successive retractor phases. When possible, in the same experiments, we also stimulated GPR in the absence of MCN1 current injection to verify the results obtained under normal physiological conditions.

Data acquisition and analysis. Data were acquired by means of analog (chart recorder, MT-95000 or Everest models; Astro-Med, West Warwick, RI) and digital (data acquisition hardware/software, Spike2; Cambridge Electronic Design, Cambridge, UK) systems. Digitized data were sampled at ~5 KHz. Data analysis was facilitated with a custom-written program for Spike2, "The Crab Analyzer," which determines the activity levels and burst relationships of individual neurons (available at http://www.uni-ulm.de/~wstein/spike2/index.html).

The burst duration of a neuron was defined as the duration (in seconds) between the first and last action potentials in an impulse burst. The firing frequency of a neuron was calculated by dividing the number of action potentials in an impulse burst minus one by the burst duration. The cycle period of the gastric mill rhythm was defined as the duration (in seconds) between the onset of two successive impulse bursts generated by the LG neuron. Unless otherwise stated, each datum in a data set corresponds to the mean of 10 consecutive gastric mill cycles. Data are presented as the mean ± SE, except where noted as mean ± SD. Comparisons between most data sets were made using a paired Student's *t* test. We analyzed data sets in which we compared activity levels before, during, and after GPR stimulations with a repeated measures ANOVA (RM-ANOVA). Statistical tests were performed with SigmaStat 3.0 and SigmaPlot 8.0 software (SPSS, Chicago, IL). Figures were made with Adobe Photoshop (Adobe Systems, San Jose, CA) and PowerPoint (Microsoft, Seattle, WA) graphics programs.

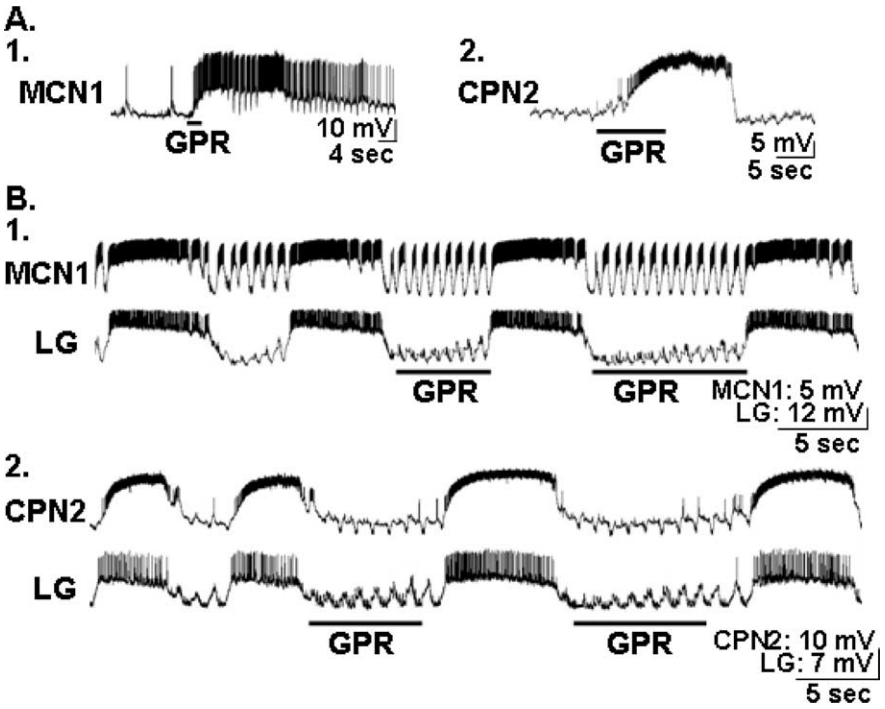

Figure 2. The GPR neuron fails to excite MCN1 and CPN2 during the VCN-triggered gastric mill rhythm. ***A***, Before VCN stimulation, in the absence of a gastric mill rhythm, GPR stimulation (bars, 5 Hz) excited both MCN1 and CPN2. ***B***, During the VCN-triggered gastric mill rhythm, GPR stimulation (bars, 5 Hz) failed to alter the activity of either MCN1 (1) or CPN2 (2). Note that, despite the loss of GPR influence on MCN1 and CPN2 in the CoG during the gastric mill rhythm, GPR stimulation still prolonged the gastric mill retractor phase (LG interburst). The MCN1 recording in ***A*** and ***B*** are from the same preparation. The CPN2 recordings in both panels are also from the same preparation but a different preparation from those in which MCN1 was recorded.

Results

MCN1 and CPN2 are necessary and sufficient to drive the VCN- and GPR-elicited gastric mill rhythms

Nearly all of the projection neurons that innervate the STG in *C. borealis* originate in the CoGs (Coleman et al., 1992). In this species, there are ~20 such projection neurons, each of which is likely present as a single neuron in each CoG (Coleman et al., 1992). Four of these projection neurons, including MCN1, MCN5, MCN7, and CPN2, are identified, and at least some of their actions on the STG are characterized (Coleman and Nusbaum, 1994; Norris et al., 1994, 1996; Coleman et al., 1995; Blitz et al., 1999; Stein et al., 2007). All four of these CoG neurons are excited, albeit to different extents, by stimulation of either the VCNs or GPR (Beenhakker et al., 2004; Blitz et al., 2004). Specifically, MCN1 and CPN2 respond to VCN and GPR stimulation with higher frequency firing and for a longer duration than either MCN5 or MCN7. GPR also presynaptically inhibits the axon terminals of MCN1 within the STG (Beenhakker et al., 2005). It remains to be determined whether additional projection neurons are also influenced by these sensory pathways. Nonetheless, the coactivation of MCN1 and CPN2 by either the VCNs or GPR is both necessary and sufficient to drive the resulting gastric mill rhythms (Beenhakker and Nusbaum, 2004; Blitz et al., 2004).

GPR does not influence MCN1 and CPN2 in the CoG during the VCN-triggered gastric mill rhythm

Activation of the proprioceptor GPR neuron during each retractor phase of the VCN-triggered gastric mill rhythm selectively prolonged this phase and thereby slowed the rhythm (Fig. 1*D*) (Beenhakker et al., 2005). This GPR action is mediated primarily by its presynaptic inhibition of $MCN1_{STG}$ (Beenhakker et al., 2005). However, GPR also evokes a prolonged excitation of both MCN1 and CPN2 in the CoGs (Fig. 2*A*) (Blitz et al., 2004). The contribution of these excitatory actions to the GPR influence on the VCN-triggered gastric mill rhythm was unknown. To assess this contribution, we rhythmically stimulated GPR during the retractor phase of this rhythm while recording from MCN1 and CPN2 in the CoG.

The VCN-triggered gastric mill rhythm is amenable for these experiments because it is stable for tens of minutes after VCN stimulation, and both MCN1 and CPN2 exhibit stereotyped activity patterns during this rhythm (Beenhakker and Nusbaum, 2004; Beenhakker et al., 2004). Specifically, during the VCN-triggered rhythm, MCN1 is tonically active for the duration of each protractor phase (LG neuron burst) and it fires brief, periodic bursts during each retractor phase (LG neuron interburst) (Fig. 2*B*). These brief, periodic bursts in MCN1 are time-locked to the pyloric rhythm, which is generated in the STG and feeds back via synaptic actions to the CoG projection neurons (Beenhakker and Nusbaum, 2004; Wood et al., 2004; Marder and Bucher, 2007). CPN2 is inhibited by the gastric mill retractor neuron Int1 (Norris et al., 1994) (Fig. 1*B*) and therefore was generally silent or weakly active during the retractor phase (Fig. 2*B*). During protraction, when Int1 is silent, CPN2 fires tonically (Fig. 2*B*). These features enabled a stable baseline of gastric mill rhythm-related activity in MCN1 and CPN2 to compare with the consequences of GPR stimulation.

Concomitant with its selectively prolonging the retractor phase of the VCN-triggered gastric mill rhythm, GPR stimulation prolonged the duration of pyloric-timed activity pattern of

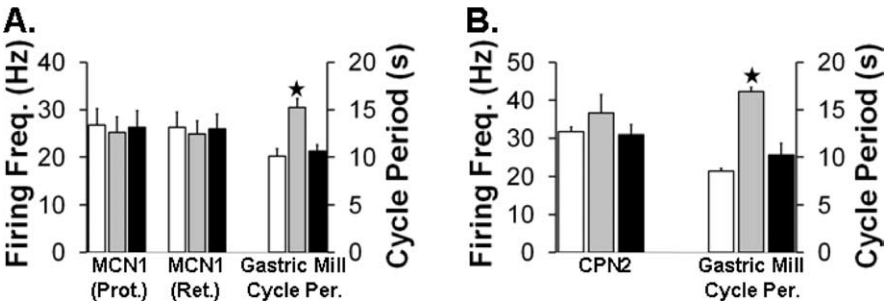

Figure 3. GPR stimulation does not alter the firing frequency of MCN1 and CPN2 during the VCN-triggered gastric mill rhythm. ***A***, GPR stimulation during the retractor (Ret.) phase of the VCN-triggered rhythm did not alter the MCN1 firing frequency during either that phase ($p = 0.47$; $n = 5$) or the subsequent protractor (Prot.) phase ($p = 0.08$; $n = 5$; RM-ANOVA), despite its ability to prolong the gastric mill cycle period (Per.) at these times (*$p < 0.05$; $n = 5$). ***B***, GPR stimulation during the retractor phase did not activate CPN2 during that phase ($n = 4$), nor did it alter the CPN2 firing frequency during the subsequent protractor phase ($p = 0.35$; $n = 4$; paired *t* test). Pre-GPR (white), during-GPR (MCN1, gray), or protraction immediately after-GPR (CPN2, gray), and post-GPR (black) stimulation during the VCN-triggered gastric mill rhythm are shown.

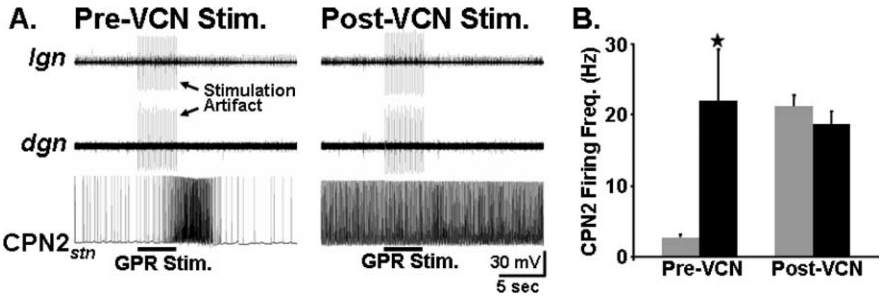

Figure 4. The loss of GPR excitation of CPN2 does not result from a synaptic action in the STG. ***A***, Left, With transmitter release selectively suppressed in the STG by superfusion of low Ca^{2+} saline (see Materials and Methods), GPR stimulation (Stim.) (5 Hz) excited CPN2 in the CoGs (pre-GPR, 5.3 Hz; post-GPR, 43.5 Hz). Note that neither the LG (*lgn*) nor DG (*dgn*) neurons were active, resulting from the suppression of transmitter release in the STG. CPN2 was recorded intra-axonally in the stn nerve ($CPN2_{stn}$) near the STG (Beenhakker and Nusbaum, 2004). Right, Under the same conditions, VCN stimulation elicited a long-lasting activation of CPN2. Here, the CPN2 response was tonic activity because of the lack of gastric mill-timed feedback from the STG (note the lack of activity in LG and DG). At this time, GPR stimulation (5 Hz) did not alter the CPN2 firing frequency (pre-GPR, 23 Hz; post-GPR, 22.5 Hz). Both panels show recordings from the same preparation. Most hyperpolarized Vm, $CPN2_{stn}$, −57 mV. ***B***, Under conditions where transmitter release was suppressed in the STG with low Ca^{2+} saline, GPR stimulation consistently excited CPN2 when the VCNs had not been recently stimulated (*$p < 0.05$; $n = 6$). However, after VCN stimulation, GPR no longer influenced CPN2 activity ($p = 0.09$; $n = 6$). Gray bars, Pre-GPR stimulation; black bars, post-GPR stimulation.

MCN1 (Fig. 2*B*). However, during this time, GPR stimulation did not alter the intraburst firing frequency of MCN1 (pre-GPR, 26.3 ± 3.2 Hz; during GPR, 24.9 ± 2.8 Hz; post-GPR, 26.0 ± 3.1 Hz; $p = 0.08$, $n = 5$, RM-ANOVA) (Fig. 3*A*). The MCN1 activity level during each subsequent protractor phase was also unchanged (pre-GPR, 26.7 ± 3.4 Hz; during GPR, 25.2 ± 3.3 Hz; post-GPR, 26.3 ± 3.5 Hz; $p = 0.471$, $n = 5$, RM-ANOVA) (Fig. 3*A*).

There was also no change in CPN2 activity during these GPR stimulations. For example, GPR stimulation during the retractor phase did not activate CPN2 (Fig. 2*B*). Furthermore, during the subsequent protractor phase, the CPN2 firing frequency was unchanged (pre-GPR, 31.7 ± 1.3 Hz; post-GPR, 36.7 ± 4.8 Hz; $p = 0.35$, $n = 4$, paired *t* test) (Fig. 3*B*). This loss of GPR influence on MCN1 and CPN2 in the CoG occurred despite the fact that GPR consistently excited both projection neurons before and after each rhythm ($n = 5/5$) (Fig. 2*A*). Additionally, as indicated above, at these times, GPR also consistently prolonged the retractor phase via its presynaptic inhibition of $MCN1_{STG}$ (Beenhakker et al., 2005).

VCN gating of GPR excitation of projection neurons occurs in the CoG

The loss of the GPR excitation of MCN1 and CPN2 during the VCN-triggered gastric mill rhythm could have resulted from events occurring in either the CoGs or STG, because the VCNs have actions at both locations (Beenhakker et al., 2004). Specifically, in addition to the VCN excitation of MCN1 and CPN2 in the CoGs, VCN stimulation directly influences the pyloric rhythm in the STG (Beenhakker et al., 2004) (R. Seaman, L. Zhang, and M.P.N., unpublished observation). To determine whether the STG was the site at which the GPR actions in the CoGs were gated out, we selectively suppressed transmitter release in the STG by superfusing it with low Ca^{2+} saline (see Materials and Methods). Doing so eliminated both the transmitter-mediated VCN actions in the STG and, by suppressing the gastric mill and pyloric rhythms, eliminated the normal rhythmic feedback from the STG to MCN1 and CPN2 (Coleman and Nusbaum, 1994; Norris et al., 1994; Wood et al., 2004).

With transmitter release selectively suppressed in the STG, separate stimulation of both GPR and VCN still effectively activated MCN1 and CPN2 (Fig. 4). Moreover, the VCN stimulation still triggered a long-lasting activation of MCN1 (data not shown) and CPN2, although in this case, their activity was tonic instead of gastric mill rhythm-timed because of the aforementioned suppression of the STG rhythms (Fig. 4*A*). Despite the loss of STG feedback to MCN1 and CPN2 and the resulting change in their activity pattern, the GPR excitation of these projection neurons was still effectively suppressed by VCN stimulation (Fig. 4). For example, the CPN2 firing frequency was consistently unchanged by GPR stimulation under this condition (pre-GPR, 21.1 ± 1.6 Hz; during GPR, 19.6 ± 1.9 Hz; post-GPR, 18.6 ± 1.9 Hz; $p = 0.09$, $n = 6$, RM-ANOVA) (Fig. 4*B*). This loss of GPR influence was also the case for the MCN1 firing frequency (pre-GPR, 17.3 ± 0.5 Hz; during GPR, 16.3 ± 1.1 Hz; post-GPR, 17.5 ± 0.7 Hz; $p = 0.55$, $n = 4$, RM-ANOVA). Thus, the VCN gating of GPR excitation occurred anterior to the STG and likely occurred in the CoGs.

VCN gating of GPR actions in the CoG is not a ceiling effect of projection neuron firing frequency

We noted that the MCN1 and CPN2 firing frequencies that occurred during their response to GPR stimulation were comparable with their firing rates after VCN stimulation (Figs. 3, 4). We therefore tested the hypothesis that the increased firing frequency of the CoG projection neurons during the VCN-triggered gastric mill rhythm caused a maximal firing frequency in these neurons that prevented any additional response to a subsequent GPR stimulation. Thus, at times when there had not been a recent VCN stimulation nor was there an ongoing gastric mill rhythm,

we depolarized MCN1 via intracellular current injection so that it fired at a frequency comparable with its firing frequency during the VCN-triggered gastric mill rhythm. As shown previously, the resulting level of MCN1 activity drove the gastric mill rhythm (Fig. 5) (Coleman and Nusbaum, 1994; Wood et al., 2004). While maintaining MCN1 at this elevated firing rate, we stimulated GPR and determined the MCN1 response. Despite the high level of activity induced by continual depolarizing current injection into MCN1, and the rhythmic feedback from the resulting MCN1-elicited gastric mill rhythm, GPR stimulation routinely increased the MCN1 firing frequency (pre-GPR, 29.0 ± 1.7 Hz; during GPR, 38.8 ± 2.2 Hz; $p < 0.05$, $n = 4$) (Fig. 5).

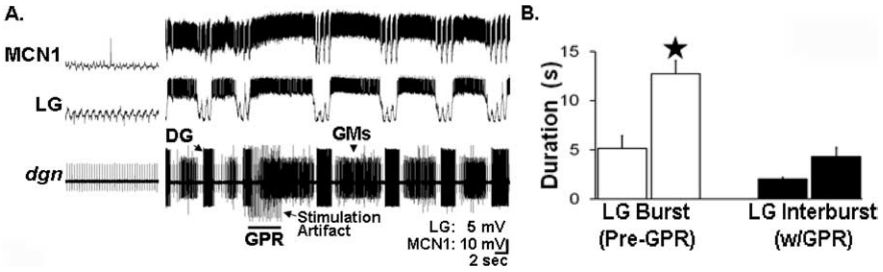

Figure 5. The failure of GPR to excite MCN1 is not a consequence of a ceiling effect in the MCN1 firing frequency. ***A***, In the absence of a gastric mill rhythm, MCN1 was injected with sufficient depolarizing current to enable its firing frequency (29 Hz) to be comparable with that occurring during the VCN-triggered gastric mill rhythm. This stimulation of MCN1 elicited the gastric mill rhythm, monitored here by the coordinated rhythmic bursting of the LG and gastric mill (GM) protractor neurons alternating with the rhythmic bursting of the DG retractor neuron. Despite this elevated level of MCN1 activity, brief GPR stimulation (5 Hz) still excited MCN1 and thereby prolonged the protractor (LG neuron) phase of the rhythm. Most hyperpolarized Vm: MCN1, −47 mV; LG, −64 mV. ***B***, Stimulating GPR during episodes when MCN1 was depolarized to fire at levels comparable with its activity in response to VCN stimulation consistently resulted in a prolongation of the protractor phase (*$p < 0.05$; $n = 3$) and no change in the retractor phase duration ($p = 0.07$; $n = 3$).

We noted that, during the MCN1-elicited gastric mill rhythm, GPR stimulation not only enhanced MCN1 activity, but it selectively prolonged the protractor phase (pre-GPR, 5.2 ± 1.3 s; during GPR, 12.7 ± 1.4 s; $p < 0.05$, $n = 3$) (Fig. 5). At these times, the retractor phase duration was unchanged (pre-GPR, 2.1 ± 0.2 s; during GPR, 4.3 ± 0.9 s; $p = 0.13$, $n = 3$, paired t test). In contrast, GPR stimulation causes a selective prolongation of the retractor phase during either the VCN-triggered gastric mill rhythm or the MCN1-elicited rhythm after removal of the CoGs (Fig. 1*D*) (Beenhakker et al., 2005).

We performed a comparable experiment to determine whether the elimination of GPR excitation of CPN2 after VCN stimulation resulted from CPN2 firing at a maximal firing frequency. As we did with MCN1, CPN2 was depolarized to achieve a firing rate approximating the 25–30 Hz frequency at which it fired during the VCN-triggered gastric mill rhythm (Fig. 3) (Beenhakker and Nusbaum, 2004). Unlike tonic stimulation of MCN1, tonic stimulation of CPN2 did not activate the gastric mill rhythm (Norris et al., 1994). As was the case for MCN1, despite the imposed increase in baseline CPN2 firing frequency, GPR stimulation in the absence of previous VCN stimulation consistently increased the CPN2 activity level (pre-GPR, 25.5 ± 1.9 Hz; immediately after GPR, 44.5 ± 3.4 Hz; $p < 0.05$, $n = 4$) (Fig. 6). In these experiments, CPN2 firing frequency was measured during the sustained, nonpyloric burst that followed each GPR stimulation. Thus, the failure of GPR to excite MCN1 and CPN2 after VCN stimulation was not the result of an inability of either MCN1 or CPN2 to fire at higher frequencies.

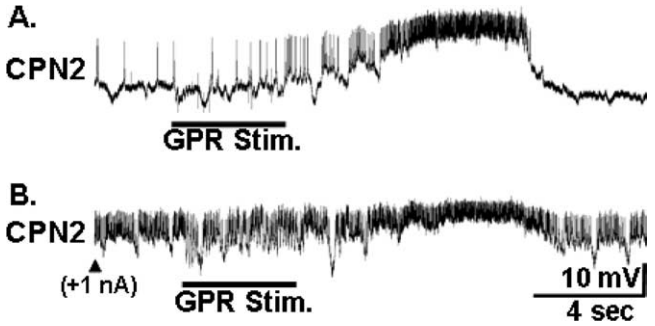

Figure 6. The failure of GPR to excite CPN2 is not a consequence of a ceiling effect in the CPN2 firing frequency. ***A***, CPN2 was excited by GPR stimulation (Stim.) in the absence of previous activation of the VCN neurons. This GPR stimulation caused CPN2 to depolarize and produce a prolonged action potential burst. ***B***, CPN2 was depolarized by constant amplitude intracellular current injection to produce an activity level (28 Hz) comparable with that observed during the VCN-triggered gastric mill rhythm (Beenhakker and Nusbaum, 2004). Under this condition, GPR stimulation still excited CPN2. ***A*** and ***B*** are from the same preparation.

VCN gating of GPR actions in the CoGs maximizes the GPR influence on MCN1 in the STG

We next aimed to determine the consequences for the VCN-triggered gastric mill rhythm if the GPR excitation of MCN1 and CPN2 in the CoGs was not gated out during this rhythm. To this end, during the VCN-triggered gastric mill rhythm, we simultaneously depolarized MCN1 with intracellular current injection (range in different experiments, 0.5–2.0 nA) and stimulated GPR, during a succession of retractor phases (Fig. 7). We depolarized MCN1 such that its firing rate increased by the same amount as occurred during GPR stimulation in the experiments shown in Figure 5. In those experiments, we stimulated GPR at times when MCN1 was depolarized to fire at the same levels as occurred during VCN stimulation. In most of these experiments, before depolarizing MCN1, we tested the MCN1 response to GPR stimulation in the absence and presence of the VCN-triggered gastric mill rhythm. As reported above, MCN1 was consistently excited by GPR in the former condition (data not shown). Also similar to our previous results, the MCN1 firing frequency was not altered under the latter condition (pre-GPR, 21.2 ± 2.2 Hz; during GPR, 19.1 ± 2.8 Hz; $p = 0.18$, $n = 5$) (Fig. 7*A*). Additionally, these GPR stimulations did increase the gastric mill cycle period (pre-GPR, 10.2 ± 0.4 s; during GPR, 12.8 ± 0.7 s; $p < 0.01$, $n = 5$) by selectively prolonging the retractor phase of this rhythm (pre-GPR, 5.3 ± 0.4 s; during GPR, 8.1 ± 0.6 s; $p < 0.01$, $n = 5$) (Figs. 7*A*, 8*A*). There was no change in the protractor phase (LG burst) duration (pre-GPR, 4.9 ± 0.1 s; during GPR, 4.6 ± 0.3 s; $p = 0.29$, $n = 5$) (Fig. 8*A*).

During the VCN-triggered gastric mill rhythm, directly depolarizing MCN1 increased its firing frequency from 18.5 ± 1.3 to 29.4 ± 2.2 Hz ($p < 0.01$; $n = 7$) (Fig. 7*B*). When this depolarization was paired with GPR stimulation, no change in the gastric mill cycle period occurred (pre-GPR, 9.1 ± 0.6 s; during GPR, 9.3 ± 0.6 s; $p = 0.18$, $n = 7$) (Figs. 7*B*, 8*B*). There was also no change in either the protractor phase (LG burst duration) (pre-GPR, 4.5 ± 0.4 s; during GPR, 4.5 ± 0.3 s; $p = 0.92$, $n = 7$) or retractor phase (LG interburst duration) (pre-GPR, 4.6 ± 0.2 s; during GPR, 4.8 ± 0.3 s; $p = 0.23$, $n = 7$) (Fig. 8*B*).

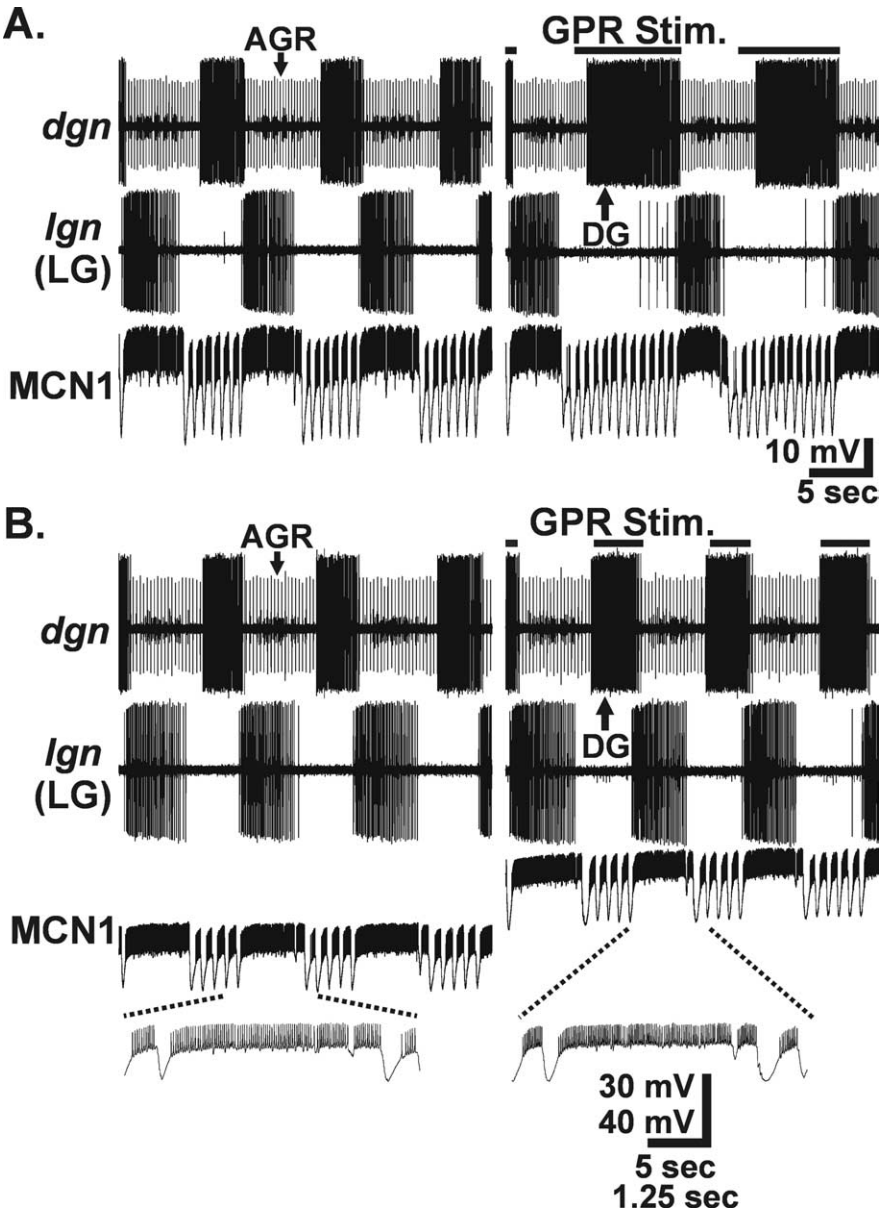

Figure 7. Artificially increasing the MCN1 firing frequency during GPR stimulation eliminates the GPR-mediated prolongation of the gastric mill retractor phase during the VCN-triggered gastric mill rhythm. ***A***, Left, Rhythmic VCN stimulation triggered a long-lasting excitation of CPN2 (data not shown) and MCN1. This MCN1 and CPN2 activity elicited the gastric mill rhythm, represented by the alternating bursting activity of LG and DG. Right, During this VCN-triggered gastric mill rhythm, GPR stimulation (bars, 5 Hz) prolonged the gastric mill cycle period by selectively increasing the retractor (LG interburst) phase. The tonically active unit in the *dgn* corresponds to the activity of the anterior gastric receptor (AGR) neuron. AGR is a muscle tendon proprioceptor neuron that is spontaneously active in the isolated STNS (Combes et al., 1995; Smarandache and Stein, 2007). Most hyperpolarized Vm: MCN1, −44 mV. ***B***, Left, VCN stimulation triggered the gastric mill rhythm, during which the MCN1 firing frequency was 22.5 Hz. Most hyperpolarized Vm: MCN1, −56 mV. Right, During the ongoing gastric mill rhythm, GPR stimulation (bars, 5 Hz) was paired with intracellular depolarizing current injection in MCN1 (+1 nA), which increased the MCN1 firing frequency to 32.5 Hz (see expanded time scale for MCN1 recording during one protractor phase pre-MCN1 and during-MCN1 depolarization). Most hyperpolarized Vm: MCN1, −31 mV. Note that, unlike in ***A***, there was no change in the gastric mill rhythm cycle period or the duration of either phase. All recordings were from the same preparation.

Discussion

In this paper, we have shown that there are focal, state-dependent actions of an identified proprioceptor neuron on the projection neurons responsible for the activation of a rhythmically active motor circuit (Fig. 9). Specifically, during the mechanosensory VCN-triggered gastric mill rhythm, the proprioceptor GPR neuron did not alter the activity of MCN1 and CPN2 in the CoGs (Fig. 9*B*). When the gastric mill rhythm has not been activated recently by the VCNs, the GPR neuron excites MCN1 and CPN2 and thereby activates the gastric mill rhythm (Fig. 9*A*) (Blitz et al., 2004). The GPR neuron also, separately, selectively prolongs the gastric mill retractor phase via presynaptic inhibition of $MCN1_{STG}$ (Beenhakker et al., 2005). Our experiments further established that this gating out of the GPR actions in the CoGs during the VCN-rhythm occurs anterior to the STG, which enables GPR to continue having synaptic influences within the STG that regulate the gastric mill rhythm, such as its inhibitory action on $MCN1_{STG}$.

The gating out of GPR actions on projection neurons that regulate CPG circuits constitutes a new locus for the gating of sensory information to motor circuits. Previous studies of such gating focused on mechanisms involving the inhibition or enhancement of afferent input directly onto CPG elements or motor neurons, often involving a phasic regulation of the incoming sensory information (El Manira et al., 1997a; Nusbaum et al., 1997; Buschges and El Manira, 1998; DiCaprio, 1999; Evans et al., 2003; Frost et al., 2003; Rossignol et al., 2006). Because the GPR excitation of the projection neurons was gated out when the gastric mill rhythm had recently been activated by a distinct sensory pathway, this gating event ensured that the elicited motor pattern was not altered by changing the firing rate and/or pattern of the activated projection neurons. As we showed in this paper, such alterations result in qualitative changes in the gastric mill rhythm. Similarly, recent work in the leech demonstrated that the type of behavior evoked by a given sensory stimulus is determined by the constellation of activated projection neurons, and the resulting behavior could be biased by the activity levels of a single projection neuron (Briggman et al., 2005).

The cellular and/or synaptic mechanisms that underlie the gating out of the GPR-mediated excitation of MCN1 and CPN2 remain to be determined. Given that the VCNs have synaptic actions in the STG as well as the CoGs (Beenhakker et al., 2004), it was possible that the VCN-mediated elimination of GPR actions on the CoG projection neurons occurred in either location. For example, there might have been a suppression of GPR spike propagation through the STG, so that the GPR spikes could not reach the CoGs. Focal regulation of sensory spike propagation within central ganglia is well documented in the molluscan nervous system (Evans et al., 2003; Frost et al., 2003) and also appears to occur in the cat spinal cord (Lomeli et al., 1998; Rudomin et al., 2004). However, our results indicate that the VCN gating of GPR actions in the CoGs

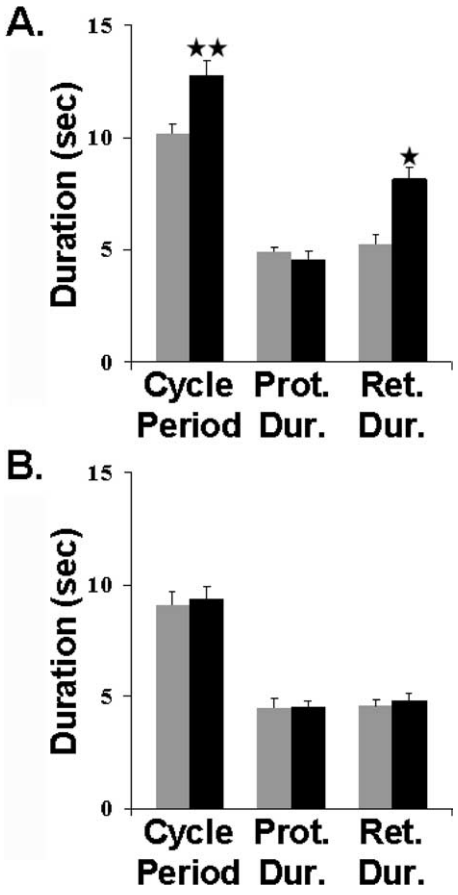

Figure 8. Artificially increasing the MCN1 firing frequency during GPR stimulation consistently prevents GPR from prolonging the retractor phase of the VCN-triggered gastric mill rhythm. ***A***, As shown previously, during an ongoing VCN-triggered gastric mill rhythm, stimulating GPR increased the gastric mill cycle period (**$p < 0.01$; $n = 5$) by selectively prolonging the retractor (Ret. Dur.) phase (LG interburst) of the VCN-gastric mill rhythm (**$p < 0.01$; $n = 5$). The protractor (Prot. Dur.) phase (LG burst) was not altered by these stimulations ($p = 0.29$; $n = 5$). Gray bars, Pre-GPR stimulation; black bars, during GPR stimulation. ***B***, During times when the MCN1 firing frequency was artificially increased by intracellular depolarizing current injection during the VCN-triggered gastric mill rhythm, GPR stimulation no longer altered the gastric mill cycle period ($p = 0.18$; $n = 6$), nor did it change either the retractor phase ($p = 0.23$; $n = 6$) or protractor phase ($p = 0.92$; $n = 6$). Gray bars, Pre-MCN1 depolarization; black bars, during MCN1 depolarization.

persisted when neurotransmitter release was selectively suppressed in the STG.

We also eliminated two other possible mechanisms that might have contributed to the VCN gating of GPR actions. First, this gating effect was not a secondary consequence of the introduction of gastric mill rhythm-timed feedback to MCN1 and CPN2 from the STG after VCN stimulation. Second, we eliminated the possibility that there was a VCN-mediated ceiling effect in the MCN1 and CPN2 firing rates. This VCN gating action was also not a necessary consequence of any recent modulatory action on these projection neurons because, during rhythmic GPR stimulation that drives the gastric mill rhythm, each subsequent GPR stimulation continues to enhance the activity of MCN1 and CPN2 (Blitz et al., 2004). It remains to be determined whether other pathways that activate a comparable gastric mill rhythm, such as stimulation of the inferior ventricular nerve neurons, also gate this GPR action (Christie et al., 2004).

There are additional presynaptic and postsynaptic mechanisms that remain to be tested. For example, the VCNs might cause a long-lasting presynaptic inhibition of GPR transmitter release in each CoG and/or a postsynaptic occlusion of the GPR actions on MCN1 and CPN2. There is precedence for both possibilities. Presynaptic inhibition of sensory axon terminals commonly occurs in sensorimotor systems. These actions are often mediated by relatively short-lasting, ionotropic mechanisms (El Manira and Clarac, 1994; Rudomin, 2002; Frost et al., 2003; Nusbaum and Contreras, 2004), but metabotropic presynaptic actions in sensory neurons also occur (Pieroni and Byrne, 1992; Parker and Grillner, 1996; El Manira et al., 1997b). Within the STNS, relatively short-lasting presynaptic inhibition focally regulates transmitter release from MCN1 within the STG (Coleman and Nusbaum, 1994; Beenhakker et al., 2005).

The possibility of a postsynaptic occlusion of GPR actions on these projection neurons in the CoG is supported by the presence of a comparable occlusion of modulatory action, involving bath-applied neuromodulators, in pyloric circuit neurons in the *C. borealis* STG (Swensen and Marder, 2000, 2001). This previously documented example of occlusion in the STG results from the convergent activation, by five different neuropeptides and a muscarinic cholinergic agonist that each bind to different receptors, of the same voltage-dependent ionic current (Swensen and Marder, 2000, 2001).

The relative influence on the gastric mill rhythm of the GPR-mediated excitation of MCN1 in the CoG and its inhibition of $MCN1_{STG}$ remains to be determined. It is noteworthy, however, that the GPR inhibition of MCN1 in the STG appears to reduce, but not eliminate, MCN1 transmitter release. This supposition is based on the fact that GPR stimulation slows but does not suppress the MCN1-elicited gastric mill rhythm (Beenhakker et al., 2005), whereas direct termination of MCN1 activity immediately terminates this rhythm (Bartos and Nusbaum, 1997). The gating out of GPR excitation in the CoGs thus appears to ensure the effectiveness of GPR inhibition of $MCN1_{STG}$ in regulating the gastric mill retractor phase duration. In support of this suggestion, we showed that GPR no longer slowed the gastric mill rhythm when the MCN1 firing rate was increased, by direct current injection, in conjunction with GPR stimulation during the VCN-triggered gastric mill rhythm.

There are several different versions of the gastric mill rhythm in addition to those resulting from GPR and VCN stimulation (Coleman and Nusbaum, 1994; Christie et al., 2004; Wood et al., 2004; Saideman et al., 2007a,b). Therefore, the GPR influence on the gastric mill rhythm may well take on additional distinct forms or, as shown recently, can have comparable actions that likely result from distinct synaptic mechanisms (DeLong and Nusbaum, 2006). Further elucidating the state-dependent actions of identified sensory neurons, and their underlying mechanisms, will likely reveal events that resonate with comparable events in other sensorimotor systems.

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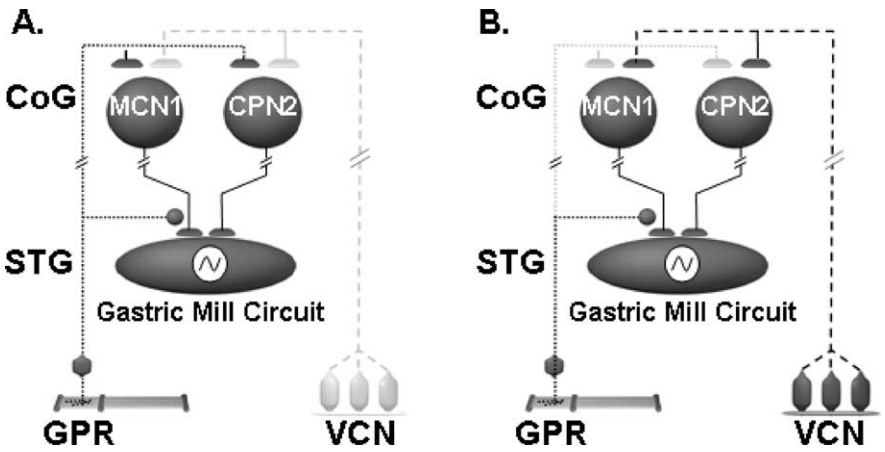

Figure 9. Schematic of the GPR proprioceptor influence on the gastric mill system in the absence and presence of the VCN mechanosensory influence on this system. ***A***, When there has not been a recent activation of the VCN neurons (pale labeling) and there is no gastric mill rhythm in progress, GPR stimulation (dark labeling) causes a relatively long-lasting excitation of MCN1 and CPN2 in the CoG, leading to the activation of the gastric mill rhythm in the STG (Blitz et al., 2004). Under this condition, GPR also causes a shorter-lasting presynaptic inhibition of $MCN1_{STG}$ (Beenhakker et al., 2005). ***B***, During the VCN-triggered gastric mill rhythm, GPR stimulation no longer excites MCN1 and CPN2 (pale labeling). However, GPR inhibition of $MCN1_{STG}$ persists during this condition (dark labeling), enabling GPR to continue providing rhythmic regulation of the gastric mill rhythm. T-bars, Synaptic excitation; filled circles, synaptic inhibition; sine wave, gastric mill rhythm activity.

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