

# **Neuromodulation of short-term synaptic dynamics examined in a mechanistic model based on kinetics of calcium currents**

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# Neuromodulation of short-term synaptic dynamics examined in a mechanistic model based on kinetics of calcium currents

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## Abstract

Network plasticity arises in large part due to the effects of exogenous neuromodulators. We investigate the neuromodulatory effects on short-term synaptic dynamics. The synapse from the lateral pyloric (LP) to the pyloric dilator (PD) neuron in the pyloric network of the crab *C. borealis* has both spike-mediated and non-spike-mediated (graded) components. Previous studies have shown that the graded component of this synapse exhibits short-term depression. Recent results from our lab indicate that in the presence of neuromodulatory peptide proctolin, low-amplitude presynaptic stimuli switch the short-term dynamics of this graded component from depression to facilitation. In this study, we show that this facilitation is correlated with the activation of a presynaptic inward current that is blocked by  $Mn^{2+}$  suggesting that it is a slowly-accumulating  $Ca^{2+}$  current. We modify a mechanistic model of synaptic release by assuming that the low-voltage-activating  $Ca^{2+}$  current in our system is composed of two currents with fast ( $I_{CaF}$ ) and slow ( $I_{CaS}$ ) kinetics. We show that if proctolin adjusts the activation rate of  $I_{CaS}$ , this leads to accumulation of local intracellular  $Ca^{2+}$  in response to multiple presynaptic voltage stimuli which, in turn, results in synaptic facilitation. Additionally, we assume that proctolin increases the maximal conductances of  $Ca^{2+}$  currents in the model, consistent with the increased synaptic release found in the experiments. We find that these two presynaptic actions of proctolin in the model are sufficient to describe its actions on the short-term dynamics of the LP to PD synapse.

**Keywords:** proctolin, graded release, short-term plasticity, central pattern generator, stomatogastric

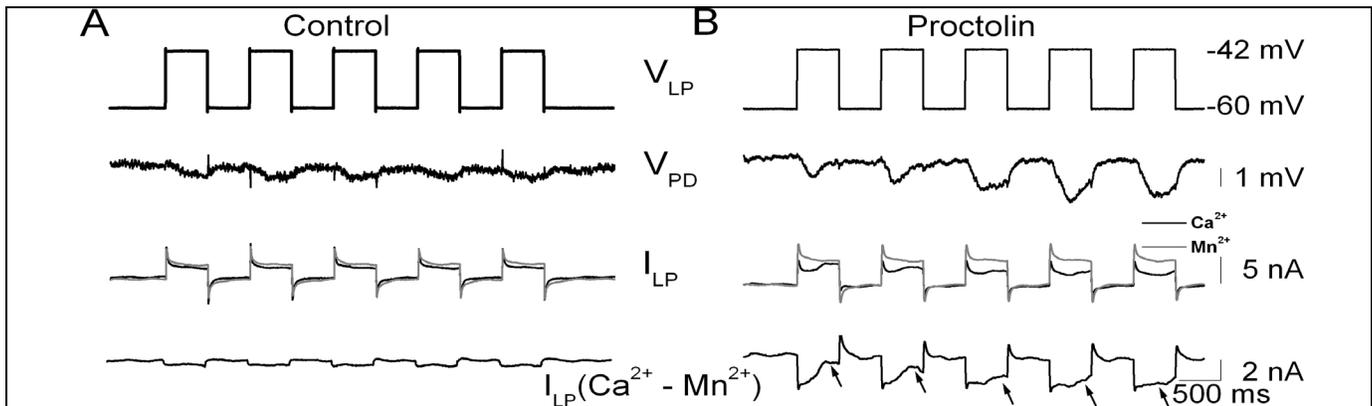
## Introduction

Neuromodulation can reconfigure a network to produce a multitude of outputs [1]. The crustacean stomatogastric nervous system (STNS) is one of the most extensively researched neural systems in studying the effects of neuromodulation. There are several reports of the actions of neuromodulators on intrinsic neuronal properties and synaptic strength in the STNS (see [2] for a review) yet there are few reports of neuromodulatory effects on the short-term dynamics of synaptic transmission. A recent report from our laboratory showed that neuromodulation can alter short-term synaptic dynamics to the extent that a depressing synapse can become facilitating [3]. Such a drastic shift in dynamics can have significant consequences for the role of that synapse in network activity. In this study, we use a combination of experiments and modeling to explore the synaptic mechanisms underlying the neuromodulator effects on the short-term dynamics of pyloric synapses.

We investigate the effects of the neuropeptide proctolin on the dynamics of the inhibitory synapse from the lateral pyloric (LP) to the pyloric dilator (PD) neuron in the rhythmic pyloric network of the crab. This synapse has both spike-mediated and non-spike-mediated (graded) components and is the only chemical feedback to the pyloric pacemaker neurons. The graded component of this synapse demonstrates short-term depression in control saline, yet, in the presence of proctolin, low-amplitude

(<30 mV) presynaptic stimulation causes the graded component of the LP to PD synapse to facilitate [3]. In contrast, with high-amplitude (>30 mV) stimuli the synapse remains depressing in the presence of proctolin, albeit enhanced in strength. We show that the switch to facilitation is correlated with the activation of a slowly activating presynaptic inward current. This inward current is blocked by  $Mn^{2+}$ , suggesting that it is a slowly-accumulating  $Ca^{2+}$  current activated by proctolin.

We propose that the switch from depression to facilitation in the LP to PD synapse is due to modifications in presynaptic  $Ca^{2+}$  current kinetics.  $Ca^{2+}$  channels can be divided into low-voltage-activated (LVA) and high-voltage-activated (HVA) channels. LVA channels such as T-type  $Ca^{2+}$  currents begin to activate  $\sim -60$  mV, peak at  $\sim -30$  mV and their amplitude decreases with further depolarization [4]. Since LVA channels have a low activation threshold, they support action potential generation, regulate subthreshold oscillatory activities, produce graded transmission and modulate spike-mediated transmission [5]. HVA  $Ca^{2+}$  currents activate  $\sim -40$  to  $-10$  mV, peak at  $\sim 0$  mV [6], and are believed to underlie spike-mediated transmitter release [6].



**Fig 1.** Facilitation of the LP to PD inhibitory postsynaptic potentials ( $V_{PD}$  trace) in proctolin is associated with the activation of a slow  $Ca^{2+}$ -like inward current. The presynaptic currents in the LP neuron are measured in  $Ca^{2+}$  saline (black) and in  $Mn^{2+}$  saline (gray) with a train of low-amplitude presynaptic voltage pulses. The difference between the two measured currents (bottom trace) shows a new inward current activated in proctolin. The amplitude of this current increases with each pulse (arrows).

LVA  $Ca^{2+}$  currents are usually inactivating. Many LVA  $Ca^{2+}$  current consists of two kinetically distinct components; one that activates/inactivates rapidly ( $I_{CaF}$ ), another that activates/inactivates slowly ( $I_{CaS}$ ) [5, 7]. We propose that the LP neuron also has two LVA  $Ca^{2+}$  currents. Additionally, we predict that proctolin not only adjusts the amplitude of these two currents but also causes  $I_{CaS}$  to activate even more slowly, which leads to accumulation of total  $Ca^{2+}$  current and facilitation of synaptic release from LP. Using a model of synaptic release, we demonstrate that these proposed effects of proctolin explain the switch in the LP to PD synapse from depression to facilitation, in response to low-amplitude presynaptic stimuli.

## Model

The synaptic release model is modified from our previous model which simulates the effect of neurotransmitter release around the readily releasable pool (RRP) [8]. Our previous model involved only a single non-inactivating LVA  $Ca^{2+}$  current. In the current model, we include two LVA  $Ca^{2+}$  currents with realistic kinetics. Specifically, the LVA  $Ca^{2+}$  currents comprise a fast ( $I_{CaF}$ ) and a slow ( $I_{CaS}$ ) activating/inactivating component which are kinetically distinct. Additionally, in the current model, the HVA  $Ca^{2+}$  current ( $I_{CaH}$ ) also plays a role in the graded release, especially with high-amplitude presynaptic voltage pulses. Briefly, the model is described as follows:

a) The  $Ca^{2+}$  currents: The total  $Ca^{2+}$  current underlying graded release is  $I_{Cag} = I_{CaF} + I_{CaS} + I_{CaH}$ , where  $I_{CaF}$  and  $I_{CaS}$  are the fast and slow LVA calcium current respectively,  $I_{CaH}$  is the HVA calcium current.

The  $I_{CaF}$  and  $I_{CaS}$  are modeled as  $I_{Ca} = G_{max} m h (V_{pre} - E_{Ca})$  and  $I_{CaH}$  is modeled as  $I_{Ca} = G_{max} m (V_{pre} - E_{Ca})$ , where  $G_{max}$  is the maximum conductance ( $G_{max}$  in  $\mu S$ : **Control**:  $I_{CaS}$  0.002,  $I_{CaF}$  0.01,  $I_{CaH}$  0.014; **Proctolin**:  $I_{CaS}$  0.008,  $I_{CaF}$  0.0175,  $I_{CaH}$  0.018),  $V_{pre}$  is the presynaptic membrane potential and  $E_{Ca}$  is the reversal potential of  $Ca^{2+}$  (fixed at 100 mV),  $m$  is the activation gate and  $h$  is the inactivation gate, the calculation of  $m$  and  $h$  is described below.

A set of differential equations are used to describe activation and inactivation gate of the above calcium currents:  $dx/dt = (x_{\infty}(V_{pre}) - x)/\tau(V_{pre})$ ,  $x = m, h$ , where  $x_{\infty}(V) = 1/(1 + \exp((V - V_x)/k))$  denotes the steady-state activation/inactivation process with parameter values  $V_x$  (in mV)  $I_{CaS}$ :  $m -35, h -27$ ;  $I_{CaF}$ :  $m -30, h -45$ ;  $I_{CaH}$ :  $m -22.5$  and  $k$  (in mV)  $I_{CaS}$ :  $m -2.0, h 10.0$ ;  $I_{CaF}$ :  $m -3.0, h 0.2$ ;  $I_{CaH}$ :  $m -6.0$ . The time constants are modeled as  $\tau(V) = \tau_1 + (\tau_h - \tau_1)/(1 + \exp(-(V + 35)/10))$  with parameter values **Control**:  $\tau_1$  (in msec)  $I_{CaS}$ :  $m 50, h 200$ ;  $I_{CaF}$ :  $m 1, h 200$ ;  $I_{CaH}$ :  $m 1$  and  $\tau_h$  (in msec)  $I_{CaS}$ :  $m 50, h 5$ ;  $I_{CaF}$ :  $m 100, h 5$ ;  $I_{CaH}$ :  $m 1$ . **Proctolin**:  $\tau_1$  (in msec)  $I_{CaS}$ :  $m 1000, h 5000$  and  $\tau_h$  (in msec)  $I_{CaS}$ :  $m 1000, h 5$ ; others unchanged.

b) The local intracellular calcium  $[Ca^{2+}]_i$ : Given the total  $Ca^{2+}$  current  $I_{Cag}$ ,  $[Ca^{2+}]_i$  is described by  $d[Ca^{2+}]_i/dt = (-\lambda I_{Cag} - [Ca^{2+}]_i)/\tau$ , where  $\lambda = 11.0 \mu M/nA$  and  $\tau = 1.0$  msec.

c) Synaptic release: The rate of change of the number of vesicles ( $N$ ) in the RRP depends on the interaction of a supply rate of new vesicles  $P(N)$  and a release rate  $R(N)$ :  $dN/dt = P(N) - R(N)$ . The supply rate is dependent on internal concentration of  $Ca^{2+}$ :

$$P(N) = \alpha \frac{[Ca^{2+}]_i + a_1}{[Ca^{2+}]_i + a_2} (N_{max} - N)$$

where  $\alpha = 0.05 \text{ msec}^{-1}$ ,  $a_1 = 2 \mu M$ ,  $a_2 = 100 \mu M$  and  $N_{max}$  is the maximum number of vesicles in the RRP (80).  $R(N)$  is given by  $R(N) = \gamma N [Ca^{2+}]_i^4$  where  $\gamma = 5 \times 10^{-7} \text{ msec}^{-1} \mu M^{-4}$ .

d) The postsynaptic response: The postsynaptic potential is given by

$C \frac{dV}{dt} = -G_{syn}(t)(V - V_{syn}) - g_m (V - V_{rest})$  where  $C$  is the membrane capacitance (1nF),  $V_{syn}$  is the synaptic reversal potential (set at  $-80$  mV),  $g_m$  is the intrinsic membrane conductance (set at  $0.0258 \mu S$ ), and  $V_{rest}$  is the membrane resting potential (set at  $-55$  mV). The postsynaptic conductance change is calculated as  $G_{syn}(t) = \int^t m G_{syn}(t') R(N) dt'$  where  $m G_{syn}$  is the miniature synaptic conductance (due to a single vesicle) which is obtained from fits to data [8].

## Biological Methods

Experiments were carried on adult male crabs (*C. borealis*) purchased from local distributors (Newark, NJ). Details of experimental measurements are identical to those described in [9]. Spontaneous activity in the pyloric network was blocked by superfusion with saline containing  $0.1 \mu M$  TTX (Biotium, Hayward, CA). (TTX does not block graded synaptic release in this system.) For measurements of synaptic resonance the presynaptic LP neuron was voltage clamped with two electrodes (TEVC) at a holding potential of  $-60$  mV and multiple low- ( $20$  mV) or high-amplitude ( $60$  mV) voltage pulses were applied. The postsynaptic response was measured in the PD neuron in current clamp mode, held at a resting potential of  $\sim -60$  mV. All data was recorded after 15 minutes of drug application or 45 minutes of wash. Data were acquired at  $5$  kHz and analyzed using the PClamp 9.2 software (Molecular Devices, Union City, CA).

## Results

To characterize short-term plasticity of the LP to PD synapse, we injected a series of multiple low-amplitude (20 mV,  $V_{LP}$  in Fig. 1) and high-amplitude (not shown) voltage pulses into the LP neuron and recorded the postsynaptic potential in the PD neuron. The experiment was performed with two different sets of treatment. First, recordings were done in both control saline (no modulators) and in the presence of  $10^{-6}$  M proctolin (Fig. 1 A, B). This proctolin concentration was used because it has been shown to best replicate the effects of proctolin release from endogenous modulatory neurons on the pyloric network [10]. Second, the experiments were performed in normal  $Ca^{2+}$  or after blocking all  $Ca^{2+}$  currents (and therefore synaptic transmission), in both control and proctolin, by substituting the  $Ca^{2+}$  (13.0 mM  $Ca^{2+}$ , the normal concentration in crab saline) with  $Mn^{2+}$  (12.9 mM  $Mn^{2+}$  and 0.1 mM  $Ca^{2+}$ ). In all conditions we made simultaneous measurements of the presynaptic current (Fig. 1,  $I_{LP}$ ) and the postsynaptic potential (Fig. 1,  $V_{PD}$ ). The difference between the presynaptic currents measured in  $Ca^{2+}$  saline and in  $Mn^{2+}$  saline is a putative calcium current (Fig. 1,  $I_{LP}(Ca^{2+} - Mn^{2+})$ ). In the control saline (with normal  $Ca^{2+}$ ), either the synaptic response showed depression (not shown, but see [3]) or no apparent synaptic plasticity was observed (Fig. 1A,  $V_{PD}$ ). In contrast, the synaptic response showed facilitation in proctolin saline (with normal  $Ca^{2+}$ ) (Fig. 1B,  $V_{PD}$ ).  $V_{PD}$  is not shown in  $Mn^{2+}$  saline because there was no synaptic transmission in  $Mn^{2+}$  saline either in control or in proctolin. The putative calcium current was small in control conditions and its amplitude showed no obvious variation among the different voltage pulses (Fig. 1A,  $I_{LP}(Ca^{2+} - Mn^{2+})$ ). However, in the presence of proctolin, this current increased in amplitude with each subsequent pulse, indicating accumulation of  $Ca^{2+}$  currents (Fig. 1B, arrows in  $I_{LP}(Ca^{2+} - Mn^{2+})$  trace). This accumulation of inward currents was correlated with the facilitation of the IPSPs in the postsynaptic PD neuron (Fig. 1B,  $V_{PD}$  trace).

In contrast with the responses measured using low-amplitude (20 mV) pulses, with high-amplitude (60 mV) pulses there was little or no accumulation of the putative  $Ca^{2+}$  current with multiple pulses in either control or proctolin. Additionally, in both conditions the IPSPs were always depressing (not shown, but see [3]).

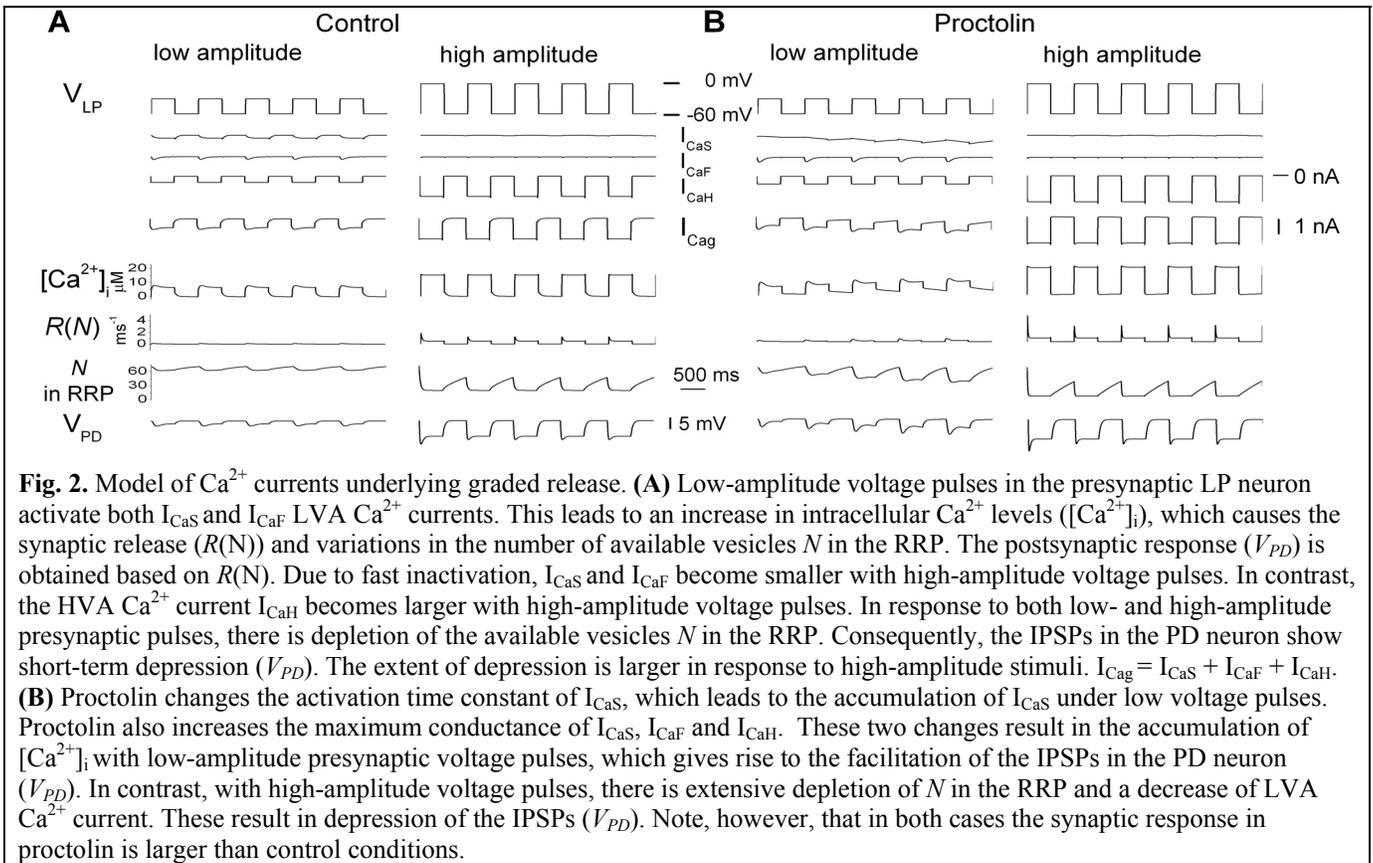
The main goal of the model is to explain how proctolin causes the short-term synaptic dynamics of the LP to PD IPSPs to switch from depression (or no change) to facilitation in response to low-amplitude presynaptic stimuli, whereas, in response to high-amplitude stimuli, the synapse remains depressing both in control and in proctolin. Additionally, the model demonstrates the enhancement of the IPSP amplitudes in proctolin, in response to any stimulus.

We incorporate the effect of proctolin in the model by modifying the activation and inactivation of the slow LVA  $Ca^{2+}$  current as well as the maximum conductances of all three  $Ca^{2+}$  currents. We propose that proctolin increases the maximum conductance of the HVA  $Ca^{2+}$  current because our experimental measurements have shown that proctolin also enhances spike-mediated release [8] which depends on this current. To simulate realistic  $Ca^{2+}$  channel kinetics, the LVA currents were set to peak at  $-30$  mV while HVA current peaked around 0 mV. There was a decrease in both LVA  $Ca^{2+}$  current amplitudes with high-amplitude voltage pulses, which resulted from fast inactivation at high presynaptic voltages (Fig. 2A, B, control LVA and HVA).

To explain the effect of proctolin on short-term synaptic dynamics we assumed that proctolin modified the kinetics of the model  $I_{CaS}$  by increasing the time constant of  $I_{CaS}$  activation from 50 to 1000 ms and the inactivation time constant (at low voltages) from  $\tau_1=200$  to  $\tau_1=5000$  ms in the presence of proctolin. Consequently, in the presence of proctolin,  $I_{CaS}$  accumulated with multiple low-amplitude presynaptic voltage pulses, as observed in the biological recordings (Fig. 1B). Furthermore, the maximum conductances of all  $Ca^{2+}$  currents were increased with proctolin compared to control (Fig. 2A, B: proctolin LVA and HVA). Due to the new kinetics of  $I_{CaS}$ , the total  $Ca^{2+}$  current  $I_{CaG}$  also accumulated

with multiple low-amplitude presynaptic voltage pulses in the presence of proctolin and its amplitude was increased (Fig. 2,  $I_{Cag}$ ).

In our model of synaptic release, the total effect of the short term synaptic plasticity is determined by the interaction between the intracellular presynaptic  $Ca^{2+}$  concentration  $[Ca^{2+}]_i$  and the number of vesicles  $N$  in the RRP. In control conditions,  $N$  is largely depleted with multiple high-amplitude voltage pulses, which results in the depression shown in the postsynaptic IPSPs in the PD neuron (Fig 2, control  $V_{PD}$ ). There is also depression in control conditions with low-amplitude voltage pulses but to a much smaller extent. Proctolin leads to the accumulation of  $[Ca^{2+}]_i$  with multiple low-amplitude voltage pulses, which in turn gives rise to the facilitation of the IPSPs in the PD neuron (Fig 2, proctolin  $V_{PD}$ ). In contrast, with multiple high-amplitude voltage pulses, there is synaptic depression which is again attributed to the depletion of the number of vesicles  $N$  in the RRP and the decrease of LVA  $Ca^{2+}$  current amplitudes.



## Discussion

Short-term synaptic dynamics such as depression and facilitation play an important role in network operation, yet their contribution to network activity is unclear. In a previous study, we produced a model of synaptic release for the LP to PD synapse in the rhythmically active crab pyloric network based on changes in intrinsic low- (LVA) and high-voltage activated (HVA)  $Ca^{2+}$  currents [8]. The current study modifies this model by incorporating realistic  $Ca^{2+}$  currents into the model to examine the role of extrinsic neuromodulation on short-term synaptic plasticity. We demonstrate that the short-term dynamics of the LP to PD synapse change from depression or stationary dynamics to facilitation in the presence of the neuropeptide proctolin. Additionally, we show that the appearance of synaptic facilitation is correlated with a presynaptic inward current that accumulates with multiple voltage pulses and is blocked if bath  $Ca^{2+}$  is substituted with  $Mn^{2+}$ . This current appears with voltage pulses from  $-60$

to  $-40$  mV, suggesting that it is a LVA  $\text{Ca}^{2+}$  current. Additionally, proctolin enhances synaptic release in response to presynaptic stimuli of any amplitude [3].

We incorporate one HVA and two LVA ( $I_{\text{CaF}}$  and  $I_{\text{CaS}}$ ) currents in our model to properly model spike-mediated (high-threshold) and graded (low-threshold) synaptic release. We then demonstrate that the actions of proctolin on the synapse can be explained by two simple mechanisms. First, we assume that proctolin enhances the maximum conductances of all presynaptic calcium currents, thus increasing release probability. Second, we demonstrate that the appearance of synaptic facilitation in the presence of proctolin can be explained if the kinetics of  $I_{\text{CaS}}$  is slowed down by proctolin. Although it is possible that the kinetics of the other  $\text{Ca}^{2+}$  currents are also changed by proctolin, the change in synaptic dynamics as well as the cumulative inward current (Fig. 1B) were sufficiently explained by the change in the  $I_{\text{CaS}}$  kinetics. With high-amplitude presynaptic stimuli, the synapse shows depression in both control saline and in the presence of proctolin [3]. Short-term synaptic depression in response to high-amplitude presynaptic stimuli in the model is due to depletion of vesicles, both in control and in proctolin, as described previously [8].

The importance of short-term synaptic dynamics in network activity has been demonstrated in computational and experimental studies [11, 12]. In addition, many studies show that synaptic dynamics are also subject to neuromodulation [13]. In the current study we used a mechanistic model of synaptic release to explore the underlying mechanisms of the effects of neuromodulation on short-term synaptic dynamics. To understand how neuromodulators reshape network output, however, it is necessary to explore how actions of the neuromodulator on synaptic and cellular components are integrated at the network level. The pyloric network could prove to be an ideal model for such a study because the actions of the neuropeptide proctolin on pyloric neurons have been incorporated into realistic biophysical models [14] and the current study provides the first detailed model of proctolin actions on pyloric synapses.

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