Computational modelling of a Central Pattern Generator

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Abstract

Central Pattern Generators (CPGs) are small, rhythmically active networks, which control simple repetitive behaviours, in both vertebrates and invertebrates. Research on CPGs aims at understanding how the periodic pattern is generated and modulated, by investigating the intrinsic and synaptic properties of the component neurons. In this study, we present a mathematical, biophysically-realistic model of the feeding CPG of the pond snail *Lymnaea stagnalis*. This network is composed of three groups of interneurons (N1, N2 and N3), which are sequentially activated and inhibited, thus providing a triphasic pattern of activity, which drives the movements of the feeding muscles (protraction, rasp and swallow). The model gives a successful description of the intrinsic properties of the individual neurons (e.g. plateau potentials and post-inhibitory rebound) and it reflects the topology of the biological network. The core of the model is a two-component circuit, composed of the neurons N1 medial and N2 ventral from the first two groups of CPG interneurons, which is capable of producing oscillations at low frequencies, when appropriately stimulated. A neuron from the third group, the N3 tonic, is not necessary for the generation of a rhythmic pattern, but it is included, since it provides a third phase of activity and it has a potentially modulatory role by exerting inhibitory effects of variable strength upon the rest of the CPG. This minimal oscillatory network is utilized in order to determine possible mechanisms by which a modulatory neuron known as Slow Oscillator affects the behaviour of the CPG. An important conclusion is that this neuron may mediate high frequency rhythms by inducing a reduction of both the protraction and swallow phases of the feeding cycle. The predictions of our model are in agreement with all known experimental results.

Introduction

Research aiming at unravelling fundamental structural and functional principles of nervous systems, often focuses on the study of small, relatively simple networks, which underlie rhythmic motor behaviours, such as breathing, feeding or swimming. Widely known as Central Pattern Generators (CPGs), in both vertebrates and invertebrates, these networks are capable of producing rhythmic electrical activity, even in the absence of phased sensory input, and they are, in general, subject to extensive neuromodulation. The fundamental question in the study of CPGs is how their rhythmic activity is generated, given the intrinsic properties of their component neurons and the synaptic connectivity between them, and how the characteristics of the generated pattern are modified as a result of modulatory input or previous experience. Among the best understood systems towards this direction is the feeding CPG of the pond snail *Lymnaea stagnalis*, which is the focus of the present work.

Feeding in *Lymnaea* is achieved through a series of repetitive movements of the feeding muscles, with which the animal manages to capture and ingest its food. Conventionally, each cycle of the feeding rhythm is divided into three phases (protraction, rasp and swallow) and it lasts from 3 to 15 seconds. Typically, each grazing session may involve hundreds of such cycles. An important characteristic of the network that controls this behaviour is that it retains its activity in the isolated ganglia (i.e. in the absence of feeding muscles), if food is present or if the activity of specific modulatory neurons is maintained, which is known as fictive feeding. Extensive electrophysiological studies of such reduced preparations have revealed a small number of premotor neurons which form a CPG (Figure 1). In each buccal ganglion, the CPG neurons (as well as the motor neurons they innervate) are conventionally divided into three groups, i.e. N1, N2 and N3, which are active during the protraction, rasp and swallow phases of the feeding cycle, respectively. In each group, further classification may be made, according to the position or the pattern of activity of each neuron, e.g. the N1 medial (N1M) from the N1 group, the N2 ventral (N2v) from the N2 group and the N3 tonic (N3t) from the N3 group. The CPG receives input from higher-order
neurons, such as the Slow Oscillator (SO), which, although not strictly necessary for the generation of the rhythm, they affect important characteristics of the motor pattern, such as its frequency or its regularity. A rich set of synaptic connections and a multitude of intrinsic properties of the component neurons, such as plateau potentials and post-inhibitory rebound (PIR), underlie the behaviour of the feeding network.

In the present study, we have developed a computational model, which summarizes the most salient features of the feeding CPG of Lymnaea. In the study of complex systems, mathematical models and computer simulations have become quite popular as a formal tool for performing precise thought experiments. Such methods are invaluable for unravelling hidden assumptions, for determining the contribution of various components of a complex system to its overall behaviour and for testing the logical consequences of various hypotheses regarding the behaviour of the system under study. In the case of biological neural networks of small size (such as a CPG), for example, they provide a “medium” for the detailed reconstruction of the system of interest. Typically, only the most relevant elements of the underlying physiology are incorporated and a series of simulations of the synthetic system are performed, which allow one to decide whether a hypothetical mechanism is sufficient to give rise to a specific type of activity and, if yes, under which conditions. Such in computo approaches may facilitate our intuition regarding the function of the network under study and they may provide testable hypotheses for inspiring future laboratory experiments. Thus, they potentially complement the standard in vivo and in vitro electrophysiological studies.

Here, we begin by modelling the intrinsic cellular properties of three crucial for the generation of the feeding rhythm neurons, i.e. the N1M, N2v and N3t. Departing from the abundant current-clamp recordings that are available, we have developed conductance-based, two-compartment models of these neurons, which are based on biologically reasonable hypotheses about the ionic mechanisms that underlie their characteristic electrical activity. At a second stage, we organize these neurons into a minimal network configuration, which reflects the topology of the biological circuit. As a result of the intrinsic cellular properties and the synaptic connectivity between the model neurons (and in accordance with previously published ideas regarding the pattern-generating ability of the feeding circuit), the model CPG is capable of producing a triphasic rhythm, when appropriately stimulated, which greatly resembles the activity of the natural network during fictive feeding. Finally, we extend the model by adding a well-known modulatory interneuron, the SO, and we show how its regulatory effects may be explained on the basis of its intrinsic properties and synaptic connections with the interneurons that form CPG.

**Methods**

**Overview of the neuronal model**

Each individual neuron of the CPG was represented with two compartments, each containing currents modelled after the formalism introduced by Hodgkin and Huxley (1956, see Figure 2). The first compartment, labelled *soma*, represents the soma and the primary neurite and it may be either passive (it may contain only a leakage current, \( I_{\text{leak}} \), as in the case of the SO) or contain slow conductances, \( I_{\text{slow}} \), which are responsible for slowly-developing, long-lasting changes of the membrane potential, such as plateau potentials and post-inhibitory rebound. In addition, it receives synaptic input, \( I_{\text{syn}} \), from other neurons in the network and it sends synaptic output, when the neuron communicates with post-synaptic units via non-spike mediated transmission, as in the case of the N2v. The compartment labelled *axon* represents the spike-generating region of the axon and, apart from the leakage current, it contains fast conductances (\( I_{\text{Na}}, I_{\text{x}} \)) for the production of action potentials. It also forms connections with post-synaptic neurons. Both compartments contain additional currents (\( I_{\text{ec,S}}, I_{\text{ec,A}} \)), which model the electrotonic coupling between them. Therefore, all neurons in the model CPG have the same structure, being different only in the properties of the
somatic compartment and the strength of the electrotonic coupling between this compartment and the axon.

A two-compartment model is the simplest possible way to represent a neuron with functionally distinct regions and, in our case, there are several reasons for assuming such a spatial separation. First of all, there is direct evidence from studies with cultured neurons, that different parts of the cell exhibit different activities. For example, the inability of isolated N2v somata to generate plateau potentials suggests that the conductances necessary for this functionality are located in distal parts of the cell, which are lost during the isolation procedure. Second, the action potentials which are recorded from the soma are small in size (typically, they do not significantly exceed the 0mV barrier), which suggests that what is recorded at the soma is the electrotonic remnants of spikes generated in different parts of the neuron. Finally, the electrical activity recorded at the soma often appears to consist of an underlying depolarization, upon which a train of full or truncated spikes is superimposed. This characteristic class of recordings may be straightforwardly reproduced in computo by assuming that the two elements (underlying depolarization and spikes) that compose this picture are located at spatially distinct, but electrotonically connected, regions of the cell, which suggests that perhaps a similar functional separation actually exists in the real neuron. Functional variations between different parts of a typical invertebrate (unipolar) neuron have long been suspected in the case of the stomatogastric ganglion and modellers of this system often use a multi-compartment approach to represent its various component neurons.

Mathematical framework

The equation for the conservation of current for the somatic compartment takes the following form:

\[ \tau_S \frac{dV_S}{dt} + i_{\text{leak},S} + i_{\text{slow},S} + i_{\text{ec},S} + i_{\text{syn}} = i_{\text{inj}} \]

where \( V_S \) is the membrane potential of the soma and \( i_{\text{inj}} \) is the current injected via an electrode. \( \tau_S \) is the passive time constant of the membrane and it equals \( C_S / g_{\text{leak},S} \), where \( C_S \) and \( g_{\text{leak},S} \) are the membrane capacitance and the leakage conductance for the soma, respectively.

The leakage current is equal to \( V_S - E_{\text{leak}} \), where \( E_{\text{leak}} \) is the reversal potential of this current.

\( i_{\text{slow}} \) represents the sum of all the slow, located at the soma conductances and its composition varies among different neurons. It has the following general form:

\[ i_{\text{slow}} = \sum_j \gamma_j k_j^p l_j^q (V_S - E_j) \]

where \( k_j \) and \( l_j \) are dynamic activation and inactivation variables, \( p_j \) and \( q_j \) are integers which, in general, take values from the set \( \{0,1,2,3,4\} \) and \( E_j \) is the reversal potential of the \( j_{\text{th}} \) current. \( \gamma_j \) equals \( g_j / g_{\text{leak},S} \), where \( g_j \) is the maximum conductance of the \( j_{\text{th}} \) current. The dynamic variables \( k_j \) and \( l_j \) follow first-order relaxation kinetics:

\[ \frac{dx_j}{dt} = \frac{x_{\text{eq},j} - x_j}{\tau_{x,j}} \]

where the steady-state values, \( x_{\text{eq},j} \), and the relaxation time constants, \( \tau_{x,j} \), are, in general, voltage-dependent and modelled using sigmoid or step functions.

\( i_{\text{ec},S} \) is the current due to the electrotonic coupling with the axonal compartment and it is equal to \( \gamma_{\text{ec},S} (V_S - V_A) \). \( V_A \) is the membrane potential of the axon and \( \gamma_{\text{ec},S} \) is given by the ratio \( g_{\text{ec}} / g_{\text{leak},S} \), where \( g_{\text{ec}} \) is the electrotonic coupling conductance.

\( i_{\text{syn}} \) is the total synaptic current received by the neuron and it is given by the following expression:

\[ i_{\text{syn}} = \sum_j \gamma_{\text{syn},j} s_j (V_S - E_{\text{syn},j}) \]

where \( E_{\text{syn},j} \) is the reversal potential of the \( j_{\text{th}} \) synaptic current and \( \gamma_{\text{syn},j} \) is the ratio \( g_{\text{syn},j} / g_{\text{leak},S} \), where
$g_{\text{syn},j}$ is the maximum conductance for the $j$th synaptic current. $s_j$ is a dynamic variable, which represents the activation of the $j$th synapse and it is modelled as a second-order, differential-equation formulation of the $a$-function:

$$\frac{dr_j}{dt} = \frac{r_{\infty,j} - r_j}{\tau_{\text{syn},j}}, \quad \frac{ds_j}{dt} = \frac{r_j - s_j}{\tau_{\text{syn},j}}$$

where $\tau_{\text{syn},j}$ is the characteristic time constant of the synapse. $r_{\infty,j}$ is the steady-state value of the dynamic variable $r_j$ and it switches between the values 0 and 1, when the membrane potential of the presynaptic compartment crosses a threshold value, $\theta$:

$$r_{\infty} = \begin{cases} 0, & V_{\text{pre}} < \theta \\ 1, & V_{\text{pre}} > \theta \end{cases}$$

The threshold $\theta$ is typically given the value 0mV, which ensures that synaptic activation increases only when a presynaptic spike is generated. In case that synaptic transmission is not dependent on spikes (non-spike-mediated transmission, as in the N2v case), a lower threshold value is used (e.g. -40mV), which allows even membrane depolarizations of small amplitude to trigger the activation of the synapse.

For the axonal compartment we have:

$$\tau_A \frac{dV_A}{dt} + i_{\text{leak},A} + i_{\text{Na}} + i_K + i_{\text{ec},A} = 0$$

where $\tau_A$, $V_A$ and $i_{\text{leak},A}$ are defined similarly as in the case of the somatic compartment. The quantities $i_{\text{Na}}$ and $i_K$ represent an instantaneous, persistent (i.e. non-inactivating) sodium current and a delayed-rectifier potassium current, respectively. $i_{\text{Na}}$ is equal to $\gamma_{Na} m^\infty (V_A - E_{Na})$, where the activation variable $m^\infty$ is modelled using a voltage-dependent sigmoid function, the quantity $\gamma_{Na}$ is the ratio $g_{Na}/g_{\text{leak},A}$ with $g_{Na}$ corresponding to the maximum sodium conductance, and $E_{Na}$ is the reversal potential for the sodium current. Similarly, $i_K$ is given by the expression $\gamma_K n (V_A - E_K)$, where the quantities $\gamma_K$ and $E_K$ are defined similarly to the sodium case. The dynamic variable $n$ follows relaxation kinetics:

$$\frac{dn}{dt} = \frac{n^\infty - n}{\gamma_n}$$

where the steady-state value $n^\infty$ and the relaxation time constant $\tau_n$ are voltage-dependent and modelled using sigmoid functions.

The form and parameters of $i_{\text{leak},A}$, $i_{Na}$ and $i_K$ were chosen such that the axonal compartment exhibits Class I excitability, according to Hodgkin's classification. The rationale behind this choice is that it has been observed that the firing rate of the neurons that are being modelled demonstrate smooth transitions, as in the initial stages of the N1M plateau or the termination of the post-inhibitory rebound of the N3t (see Figure 4 and 6), which suggests a Class-I-excitable firing mechanism (Figure 3).

Finally, the current due to the electrotonic coupling with the soma, $i_{\text{ec},A}$ is equal to $\gamma_{A} (V_A - V_S)$, where $\gamma_{ec,A}$ is given by $g_{\text{ec},A}/g_{\text{leak},A}$. $g_{\text{ec},A}$ is the electrotonic coupling conductance with somatic compartment.

**Simulations**

The models for the individual neurons and the final model for the CPG took the form of a system of ordinary differential equations (ODEs), which was encoded and solved using custom code written in the programming language ANSI C and the GNU Scientific Library (v. 1.6). All the simulations were realized on a Pentium IV with 512 MB of memory and the Linux operating system. For the solution of the ODEs, the `gsl_odeiv_step_rk8pd` routine was used, which implements an embedded
Runge-Kutta Prince-Dormand (8,9) algorithm. The step size was adaptive, in order to meet an absolute error tolerance of $10^{-12}$ and a relative error tolerance of 0.0.

Results

Intrinsic cellular properties

**N1M.** The first neuron we modelled was the N1M. N1M's characteristic pattern of plateauing activity (Figure 4A) begins as a train of spikes, which gradually increase in frequency and decrease in amplitude. Progressively, the membrane reaches a sustained, depolarized state at about -30 mV, upon which truncated action potentials are superimposed. In the intact nervous system, the plateau is terminated due to inhibitory input received from presynaptic neurons. Cultured N1M cells retain their ability to generate plateau potentials as a result of brief and sufficiently strong excitatory input, which is a strong indication that this is actually an intrinsic property of the neuron.

How may this characteristic pattern of electrical activity be explained? It was assumed that the activation of a strong, inward current, $I_{\text{dep}}$, is responsible for the generation of the plateau. The initial transitory phase of the plateau, which is marked by the progressive changes in the frequency and size of the action potentials reflects the slow activation of this current. As a larger proportion of the current is activated and the membrane is gradually depolarized, more current flows to the axonal compartment, which presumably explains the gradual increase in the spike frequency. An important element of this model is that $I_{\text{dep}}$ has a reversal potential of about -30 mV, i.e. the same value as that of the membrane potential, after the plateau has reached its steady-state. This condition has one very interesting consequence: as the magnitude of the depolarizing current increases, it progressively shadows all the other currents and the membrane potential moves towards a new equilibrium point, which is the reversal potential of $I_{\text{dep}}$. As a result, the size of the soma-recorded action potentials progressively decreases until the point where only small electrotonic remnants of them remain, distributed around the equilibrium potential of the depolarizing current.

What could be the nature of this hypothetical current? Although the exact current composition of the cell is not known, it is suggested that it may be an ACh-activated current, $I_{\text{ACh}}$. It has been shown that the N1M is both cholinergic and responsive to externally applied ACh and, more importantly, that the response of isolated somata to ACh has a reversal potential of about -30 mV, as required. In addition, if this is indeed the case, then a possible mechanism for the activation of the current is readily suggested, i.e. since the N1M both releases and reacts to ACh, $I_{\text{ACh}}$ may react to ACh released by N1M itself, thus initiating a positive feedback loop, which leads to the full activation of the current, as summarized in Figure 3C. Here an initial membrane depolarization above a threshold value of -40 mV triggers the release of ACh from the soma, which activates $I_{\text{ACh}}$. The induced membrane depolarization causes further release of ACh, activation of $I_{\text{ACh}}$, depolarization and so on, until the plateau is fully developed. In the model, the threshold for the release of ACh from the soma was given a rather negative value (-40 mV), because it has been shown that the plateau may develop even in the absence of action potentials, which suggests a low threshold for transmitter release.

A final assumption of the model concerns the time constant for the activation of $I_{\text{ACh}}$. It was assumed that for very negative values of the membrane potential (<-60 mV), $I_{\text{ACh}}$ is de-activated rapidly ($\tau=50\text{msec}$), while for the more positive values, the activation of the current takes place at a very slow rate ($\tau=1\text{sec}$). This separation of the activation time constant in two regimes was deemed necessary, since it makes possible the termination of the plateau by inhibitory stimuli that drive the membrane potential below -60 mV for a sufficiently long time interval.

As shown in Figure 4B, the model that implements the proposed mechanism may successfully reproduce the characteristic waveform of the N1M plateau. The reversal potential of $I_{\text{ACh}}$, $E_{\text{ACh}}$, determines the membrane potential value the plateau finally reaches, as shown in Figure 4D. Finally, an important consequence of the way the model was designed is that the firing frequency is
not affected by the magnitude of the injected current, but rather by $E_{\text{ACh}}$, which determines how much current electrotonically invades the axonal compartment and, therefore, the frequency at which the action potentials are generated. This property is summarized in Figure 4E.

**N2v.** In relation to N1M, the N2v demonstrates a more conventional firing pattern (Figure 5A). The N2v plateau consists of a significant ($\approx 40\text{mV}$) membrane depolarization, upon which truncated action potential are superimposed. The plateau terminates spontaneously approximately after $1\text{sec}$. Although cultured somata do not possess the ability to generate plateau potentials, N2v cells in the intact nervous system are capable of producing plateaus if appropriately stimulated, even when synaptic input from the rest of the network has been blocked. This is a strong indication that the plateau-generating ability of the N2v is intrinsic to the neurons, but the responsible conductances are located at distal to the soma regions, for example, in the primary neurite and its processes.

In the context of the present model, we have assumed that two slow located-at-the-soma conductances are responsible for the generation of the plateau. A relatively fast activating inward conductance $p$ is responsible for the rising phase of the plateau. The delayed activation of a second outward conductance $q$, at more depolarized levels, is responsible for the repolarization of the membrane after $\approx 1\text{sec}$. While the somatic compartment remains depolarized during the plateau, the axonal compartment is being stimulated by current entering from the soma. The spikes generated in this way are electrotonically transmitted back to the soma and recorded as attenuated action potentials on top of the plateau depolarization.

As shown in Figure 5B, the model is capable of generating plateau potentials which greatly resemble the actual recordings. An overview of a single plateau potential and the temporal changes of the underlying conductances, $p$ and $q$, is given in Figure 5C. An important characteristic of the model is that it is capable of generating a series of plateau potentials when current is constantly injected, with the frequency of bursting being directly affected by the amount of the injected current (Figure 5D).

**N3t.** The N3t has been shown to have post-inhibitory rebound properties (Figure 6A). In the intact nervous system and in the absence of externally applied stimuli, N3t fires tonically at a frequency of approximately $4\text{Hz}$. This regular activity may be temporarily interrupted by sufficiently strong inhibitory input. When the inhibition is withdrawn, the N3t excitability is transiently increased, resulting in a short burst of spikes, riding a small ($5\text{-}10\text{mV}$) plateau potential. As the excitability of the cell returns to its normal levels, the burst of spikes fades out and the cell returns to its normal mode of tonic activity.

In other neurons with PIR (e.g. the B4 motoneurons), the presence of a hyperpolarization-activated (or, more correctly, hyperpolarization de-inactivated) current, $I_h$, has been demonstrated. Departing from this experimental evidence, we have assumed that the PIR properties of N3t are due to the presence of an $I_h$-like current, located at the somatic compartment. The current is dependent upon two dynamic variables, one for the activation and one for the inactivation of the current. When the cell is at rest, $I_h$ is inactivated and its total conductance is 0. However, if inhibitory stimuli manage to drive the membrane potential below a threshold value ($-70\text{mV}$) for a sufficiently long time interval, a proportion of the inactivation is removed. After the cell is released from inhibition, the conductance of $I_h$ is non-zero and it induces a transient increase in the firing frequency of the cell. The gradual inactivation of $I_h$ at these depolarized potentials allows the cell to return back to its normal levels of tonic activity.

It may be observed (Figure 6B) that a model based on the previous mechanism manages to reproduce the most salient features of the electrical activity of the biological neuron, i.e. a small transient depolarization of the membrane as a result of PIR, upon which a train of high frequency action potentials is superimposed. The temporal changes in the conductance of $I_h$ that underlie a single PIR event in the model are depicted in Figure 6C. Stimuli of increasing amplitude trigger PIR events of increasing intensity, since they drive the membrane potential below the threshold value of $-70\text{mV}$ for a larger time interval and, therefore, they induce the de-inactivation of a larger
SO. Relatively to the previous neurons, SO was the most trivial to model. The somatic compartment of the model was assumed to be passive (it contains only a leakage current), thus firing tonically under current clamp, at a frequency determined by the magnitude of the injected current. The action potentials recorded at the soma barely exceed the $0\text{mV}$ boundary and they may be temporarily interrupted by brief inhibitory pulses (Figure 7).

Network properties

N1M→N2v interactions. After having modelled the intrinsic cellular properties of the main neurons of the feeding CPG of *Lymnaea*, we started reconstructing the network. We begun with the N1M and N2v interneurons, since it has been observed that co-cultured neurons from the N1 and N2 groups may form pairs with pattern generating abilities. In the intact nervous system, neurons of the N1 group send excitatory connections onto the N2 neurons and they receive inhibitory input from them. By implementing this pattern of reciprocal excitatory and inhibitory synapses, we managed to form a basic two-component circuit capable of producing regular oscillations, when appropriately stimulated (Figure 8), similar to those observed in culture. The sequence of events that leads to this repetitive pattern is briefly the following: Constant current injection into the N1M excites the neuron and eventually triggers the generation of a plateau potential. The activity of the N1M slowly depolarizes the N2v due to the N1M→N2v excitatory connection. This synapse was given a relatively large time constant ($\tau=200\text{msec}$), which we assumed explains the failure to observe 1:1 EPSPs onto the N2v. In addition, we gave the synapse a relatively low conductance value, which in combination with the property of the N2v neurons to oscillate at arbitrarily low frequencies (see Figure 5B), was sufficient to induce a slow depolarizing wave onto the N2v, similar to that observed in vitro and, eventually, driving the neuron beyond the threshold for the generation of a plateau. The inhibitory effect of the N2 neurons upon the N1M has been shown to be independent of the presence of spikes, which justifies our choice to assume that a) the somatic rather than the axonal compartment of the N2v model plays the role of the presynaptic region and b) the threshold for the release of transmitter is relatively low ($-40\text{mV}$). Therefore, the activation of the N2v plateau drives the N1M membrane potential below a threshold value of $-60\text{mV}$ and, since $I_{Ac}$ de-activates rapidly at such negative values, the N1M plateau terminates before the spontaneous termination of the N2v plateau, $\approx1\text{sec}$ later. If excitatory stimulus upon the N1M is still present, its plateau recovers and a new cycle is initiated.

The role of the N3t. The feeding rhythm of *Lymnaea* is conventionally divided into three phases, each of which is characterized by the fact that only a specific group of neurons (N1, N2 or N3) is active, while the remaining two are being inhibited. Consequently, a network capable of producing a triphasic rhythm must necessarily incorporate neurons from all three groups. Therefore, our next step was to introduce the N3t into our artificial network. In the intact nervous system, the N3t receives inhibitory synapses from the N1M and N2v and, in turn, sends inhibitory input onto the N1M, but not onto the N2v. The extended network that incorporates these additional elements is capable of producing a basic rhythm completed in three phases (Figure 9). The sequence of events that leads to the generation of a third phase of activity are briefly the following: The activation of the N1M by constant current injection results in the inhibition of N3t, thus interrupting its normal tonic activity for the whole of the P phase of the rhythm. The generation of the N2v plateau and the initiation of the R phase results in a second stronger phase of inhibition being imposed onto the N3t, driving the neuron below the $-70\text{mV}$ threshold for the de-inactivation of the $I_h$. The neuron remains inhibited, until the N2v plateau is spontaneously terminated. On the recovery from the R-phase inhibition, it fires a transient burst of spikes due to PIR, while gradually returning to its normal state of tonic firing. During this period of activity, it inhibits the N1M, thus delaying the recovery of its plateau. When the N1M is finally activated again, it inhibits the N3t and a second cycle is repeated. In brief, the model predicts that the role of the P- and R-phase inhibitory input upon the N3t is to interrupt its normal tonic firing, leaving only a small window of activity, which defines the S phase.
of the feeding rhythm. Moreover, the effect of the N3t inhibitory input upon the N1M suggests a potentially modulatory role for the former. In Figure 10, the current-frequency response of the three-component network is illustrated for various conductance values of the N3t→N1M inhibitory synapse. It may be observed that the basic effect of this connection is to translate the current-frequency relationship to the right, i.e. for larger conductance values, larger amounts of injected current upon the N1M are needed in order to initiate regular oscillations and achieve rhythms of a specific frequency. The amount of the S-phase inhibition upon the N1M may also be increased by increasing the firing frequency of the N3t, in which case similar results are expected. In summary, although N3t is not necessary for the generation of the rhythm, the model predicts that it may have a potentially modulatory role by affecting the response of the CPG to injected current.

Modulatory effects of the SO. In the intact nervous system, the components of the CPG receive input from higher-order modulatory interneurons. A very important neuron in this category is the SO, which is typically used to drive regular fictive patterns at high frequencies. Having a basic network capable of producing triphasic rhythms, our next step was to extend it by adding the SO, which sends excitatory input onto the N1M and N2v and receives inhibitory input from the N2v. Current injected into the SO excites the neuron, which eventually triggers an N1M plateau via its SO→N1M excitatory connection and initiates a cycle. With the transition to the R phase, the SO is inhibited (N2v→SO), but it recovers after the N2v plateau is spontaneously terminated and it induces a new cycle of network activity (Figure 11).

A striking characteristic of the SO in the intact nervous system is that it is capable of driving fast regular rhythms (period 3-6secs), in contrast to the N1M-driven ones, which are much slower (7-15secs). In Figure 12, we compare the current-frequency relationships of an N1M-driven rhythm and a SO-driven rhythm, without (case A) and with (case B) the SO→N2v excitatory synapse. Several observations may be made. First, the SO-driven rhythms (case B) may in general achieve higher frequencies than the N1M-driven ones, as in the real system. More specifically, the lowest period an N1M-driven rhythm may achieve for a reasonably high amount of injected current is ≈7sec. However, SO-driven rhythms may achieve periods as low as 3secs, as in the natural system. A second observation concerns the duration of each phase as a function of the total period of the cycle. During an N1M driven rhythm, both the duration of the P and R phases remain relatively constant. However, the duration of the S phase reduces (almost linearly) with the whole cycle period, which means that the observed changes in the duration of the cycle are basically due to changes in the duration of the S phase. On the other hand, SO-driven rhythms demonstrate a qualitatively different behaviour. First of all, the duration of the P phase is overall shorter than in the case of the N1M rhythms. For periods larger than ≈6-7sec, the situation is similar to that of the N1M-driven rhythms, i.e. the P and R phases remain constant and only the duration of the S phase changes. However, for periods less the 6 seconds, a significant reduction in the duration of the P phase is observed, while the rate at which the duration of the S phase reduces becomes smaller. Therefore, SO manages to drive faster rhythms than the N1M by inducing changes in the duration of both the P and S phases and, in general, by preserving a P phase of shorter duration.

How does SO, but not N1M, manage to have such an effect upon the feeding rhythm? The P phase of the cycle is defined as the interval between the first spike of the N1M and the termination of the N1M plateau due to R-phase inhibition. Consequently, the sooner the N2v plateau is activated, the sooner the N1M plateau terminates and the shorter the duration of the P phase. In our model, the only source of excitation upon the N2v during an N1M-driven rhythm comes from the N1M itself. However, as demonstrated precisely, injected current does not significantly affect the firing frequency of N1M and, therefore, the time interval until the activation of an N2v plateau. What is affected is how soon the N1M recovers from the S-phase inhibition, i.e. the swallow phase of the rhythm. The situation is different with the SO-driven rhythms. First, the SO firing frequency is directly affected by the injected current and, second, the N2v receives additional excitation from the SO (case B, but not case A), which accelerates its activation. It is interesting to observe that when this SO→N2v excitatory synapse is not included (case A), the frequency of the rhythm is lower.
which is indicative of the significance of this synapse.

In summary, the model predicts that the SO manages to drive fast rhythms by inducing a reduction of both the P and S phases of the rhythm, as a result of its intrinsic properties and specific connections to the neurons of the CPG. On the other hand, the N1M cannot affect the duration of the P phase, as a consequence of its characteristic plateauing mechanism, and, therefore, can only drive relatively slow rhythms.

Discussion

The feeding network of *Lymnaea stagnalis* has been the subject of extensive electrophysiological studies, which have revealed a rich set of intrinsic properties and synaptic connections between its component neurons. An abundance of intracellular recordings from the individual cells that form the network provides insight into the ionic mechanisms that govern their electrical activity and, in combination with a detailed knowledge of the topology of the network, permits various hypotheses to be made about the sequence of physiological events that underlie the observed pattern of repetitive activity. Previous attempts to integrate the available data into a coherent picture of the pattern-generating mechanism have mainly taken the form of verbal models, which (as with all models in this category) leave much space for ambiguity and hidden assumptions and make it rather difficult to precisely explore the logical consequences of the proposed physiological processes. A formal description of the feeding CPG was given by (Dunn's thesis), who used single-compartment, Hodgkin-Huxley-type models to describe the individual neurons. Although the model manages to generate a rhythmic pattern similar to the recorded ones, it fails to give a faithful representation of various intrinsic neuronal properties (e.g. the plateau potentials of N2v and N1M) or reproduce some characteristic network properties, such as the very slow rhythms that are driven by stimulation of the N1M, as opposed to the fast SO-driven ones.

In the present study, we tried to improve upon these previous attempts, having in mind the fulfilment of a set of pre-determined criteria, which the final model should satisfy:

1. The model should give a successful description of the intrinsic neuronal properties of the individual cells, such as plateau potentials or post-inhibitory rebound.
2. The synaptic connectivity of the model network should reflect the synaptic connectivity of the natural one.
3. The model should be able to explain the observed firing pattern, i.e. the correct sequence of excitation and inhibition of the individual neurons.
4. The model should be able to explain the modulatory effects of well-defined regulatory neurons, such as the SO.
5. Finally, the model should be biophysically-realistic (i.e. every element of the model should have a well-defined physiological meaning and, therefore, it should be directly or indirectly measurable), and the number of arbitrary elements should be kept to a minimum.

Typically, we departed from the level of the individual neuron and we progressively synthesized a model of the feeding network by incorporating only those elements that we considered absolutely necessary for its correct functioning. The final product is a minimal theoretical construct, which not only summarizes existing physiological data, but it also makes novel hypotheses (e.g. the plateau-generating mechanism of the N1M and the non-necessity of the N3t for the pattern generation), which could be subject to future experimental validation.

Our current view of the feeding network is summarized in Figure 13. The core of the system is a two-component circuit composed of the N1M and N2v neurons, which is capable of slow oscillations when appropriately stimulated. The crucial elements for this behaviour are the very slow induction of the N2v plateau by the N1M and the termination of the N1M plateau by the N2v.
The former is possible due to the weak N1M→N2v excitatory synapse and the property of the N2v to generate plateau potentials at arbitrarily low frequencies. The latter is possible due to the strong N2v→N1M excitation and the property of the N1M plateau to deactivate rapidly at very negative membrane potentials. The intrinsic inability to significantly affect the firing frequency of the N1M through injected current means that the duration of the P phase of the rhythm will be largely unchanged, that all possible modifications in the duration of the whole cycle will stem from how long it takes the N1M plateau to recover from the N2v inhibition and, finally, that there is a minimum whole-cycle period that may be achieved, which cannot be lower than approximately the sum of the durations of the P and R phases of the rhythm.

It should be thought that the feeding CPG consists an extension of this core circuit by the addition of the N3t. The inhibitory input upon this neuron from the rest of the network periodically interrupts its normal tonic activity, thus defining the S phase of the feeding rhythm. The fact that the N3t exerts inhibition upon the N1M suggests a potentially modulatory role for it. One can imagine the existence of modulatory pathways, which converge onto the N3t and modify the intensity of its inhibitory effects (e.g. by changing the conductance of the N3t→N1M synapse or its firing frequency), thus regulating the response of the network to external stimuli. According to this model, the N3t should be viewed as an intermediate between pattern-generating and modulatory neurons.

At the highest level of organisation, we place modulatory neurons, such as the SO. The model predicts that a reduction in the duration of the feeding cycle below the levels achieved during an N1M-driven pattern may be induced by reducing the duration of the P phase of the rhythm. In this model, the SO manages to maintain a P phase of shorter duration by accelerating the onset of the N2v plateau through its SO→N2v excitatory synapse.

Is this view of the network justified by empirical data? In fact, there is experimental evidence in support of the crucial role of the N1M↔N2v pair and the modulatory role of the N3t, on one hand, and the effect of the SO upon the CPG, on the other.

Regarding the first issue, Staras et al. (2003) have proposed that the regulation of N3t activity plays a central role in the arousal of episodes of activity in an otherwise quiescent CPG. In particular, they found that when N3t fires tonically, it suppresses the rest of the network through its inhibitory effect upon the N1M. When the firing frequency of the N3t reduces (for example, when food is presented to the system and/or the levels of hunger are increased), the network may start oscillating, the N3t being active only during the S phase of the rhythm. When the N3t is artificially suppressed, the CPG is released from inhibition and it enters a period of normal rhythmic activity. These results are in agreement with various points of the model presented here. First, they provide justification for the N3t as an intermediate modulatory/pattern-generating interneuron, possibly being the final node in a regulatory cascade of events. Second, they show that N3t is not necessary for the generation of a rhythm, since the network may operate even in its absence. Finally, these results show that, in order to have a rhythm, a functional N1M neuron is required, as well as a source of inhibition sufficient to terminate the N1M plateau at the end of the P phase, and, therefore, they suggest the importance of the reciprocal interactions between the N1 and N2 groups of neurons.

Regarding the suggested modulatory role of the SO, the observations of Elliott and Andrew (1991) are illuminating. These researchers compared the duration of each phase of the feeding rhythm to that of the whole cycle, during spontaneous, N1M-driven and SO-driven rhythms. They found that in the first two cases, the duration of the S phase changes linearly with the whole period, the changes in the duration of P phase are not significant, while the R phase remains constant. However, in the case of the faster SO-driven rhythms, both the P and S phases change significantly with the duration of the whole cycle. The R phase remains again constant. One interesting detail is that the slope of the P phase as a function of the period of the cycle is steeper than that of the S phase, when the period is less than 6 seconds, but not when it is larger than that. These observations are in good agreement with the results of our model. Moreover, the fact that the duration of the P phase during N1M-driven rhythms does not change significantly is in agreement with the idea that
injected current does not greatly affect the firing-frequency of N1M. The idea that high-frequency rhythms involve a significant reduction in the duration of the P phase is further supported by Yeoman et al. (1996), who observed that the Cerebral Giant Cells may mediate fast rhythms by inducing such a reduction. From a behavioural point of view, this result means that during fast rhythms (e.g. in an environment rich in food particles) the animal reduces the time it spends trapping food with its *radula* (tongue), probably maximizing the amount of food being ingested.

One important difference between our model and the results of the previous authors lies in the way the reduction in the duration of the P phase is induced. They have attributed this to an increase in the firing rate of N1M (directly or indirectly) via the SO→N1M excitatory connection, while we have given more importance to the SO→N2v excitatory synapse, since our model does not assume that an increase in the firing rate of N1M takes place during SO-driven rhythms. Observations supporting that such an increase actually takes place should not be seen without doubt, since they are often indirect (i.e. they are based on firing-rate changes of motorneurons, not on direct N1M recordings). However, even if this is the case, the model should be able to account for this discrepancy, if we assume that the model SO excites the N1M near its spike-generating zone (and not at the somatic compartment, as it is currently the case), being therefore able to affect the firing frequency of the neuron. **This point needs further investigation.**

Another potential point of criticism concerns the fact that the models of the individual neurons in the network were not based on voltage-clamp data, but mostly on a large set of current-clamp recordings. Therefore, the possibility that a different combination of ionic currents underlies the intrinsic properties of the real neurons cannot be excluded. Results from the dynamical systems theory have shown that neurons with different ionic currents can have similar dynamics and, conversely, neurons with similar currents can have very different behaviours. In the present case, our conclusions on the functionality of the network were based on specific neuronal properties (i.e. a well-defined response to current injection, such as the Class I excitability of the axon, the insensitivity of the N1M firing rate to somatic stimulation or the ability of the N2v for repetitive plateauing), not on the exact current content of the membrane. Therefore, as long as the model neurons (i.e. the building blocks of the circuit) exhibit the correct phenomenology (dynamics or behaviour), our speculations about how the system operates at the network level are still valid.

In conclusion, we have built a minimal model of the feeding CPG of *Lymnaea*, which summarizes much of the available physiological data and makes interesting hypotheses about the functionality of the system, both at the cellular and the network level. These hypotheses could be the subject of future experimental validation and, up to this point, independently collected empirical data are in good agreement with the predictions of the model. Considering the proposed central role of the N1/N2 oscillatory pairs for the functionality of the network, it is reasoned that further electrophysiological studies could be focused on the investigation of the exact plateauing mechanism of the N1M and N2v interneurons.
Feeding in *Lymnaea* is achieved via the repetition of a basic triphasic pattern of movements of the feeding muscles (*bucal mass*) and it is controlled by a network of interneurons, which form a Central Pattern Generator (CPG). Overview of the feeding CPG. The interneurons that compose the network are divided into three groups, i.e. *N1*, *N2* and *N3*. The most important neurons from each group (*N1 medial* or *N1M*, *N2 ventral* or *N2v*, *N3 tonic* or *N3t*) and the synapses between them are illustrated (bars indicate excitation, circles indicate inhibition). The CPG makes synaptic contact with modulatory interneurons, which although not necessary for the generation of the rhythm, affect various of its characteristics, such as its frequency or its regularity. A very important neuron of this type, the *Slow Oscillator* or SO, is also depicted. A typical example of the rhythmic electrical activity of the CPG is shown on the left. Among the illustrated intracellular recordings, only those of the SO and N2v are simultaneous. The rest have been obtained separately and appropriately scaled. The feeding cycle is divided into three phases, i.e. *protraction*, *retraction* and *swallow*, each describing a distinctive set of movements of the feeding muscles (indicators at the top). During each phase, only the neurons from a single group fire (activating corresponding sets of motorneurons and, eventually, of feeding muscles), while the rest remain inhibited (indicators at the bottom). The pattern has been generated by constant current injection into the SO (*SO-driven rhythm*). Alternatively, rhythmic activity could be induced via stimulation of the N1M (*N1M-driven rhythm*). In that case, the SO remains silent.
Figure 2: Each neuron in the network was represented as a two-compartment, conductance-based model, which assumes a spatial separation between fast (action potentials) and slow (post-inhibitory rebound, plateau potentials) processes. General structure of the neuronal model. The compartment labelled soma represents the soma and its proximal neuritic processes. It forms synaptic connections with pre-synaptic neurons ($I_{\text{syn}}$) and it may contain slow conductances ($[I_{\text{slow}}]$), which are responsible for low-amplitude, slow-developing changes of the membrane potential, such as plateau potentials or post-inhibitory rebound. The brackets illustrate the fact that $I_{\text{slow}}$ may be absent, as in the case of the SO, where the somatic compartment is completely passive. The axonal compartment contains fast conductances ($I_{\text{Na}}, I_{\text{K}}$), which are responsible for the generation of spikes. Action potentials recorded at the soma are assumed to be electrotonic remnants of events initiated in this compartment. In addition, the axon forms connections with post-synaptic neurons. An exception to this rule is the N2v, where non-spike-mediated transmission takes place. In this case, the soma acts as the presynaptic compartment, as well. Both compartments contain additional currents ($I_{\text{ec,1}}, I_{\text{ec,2}}$), which model the electrotonic coupling between them. A justification of the two-compartments approach is given in the main text.
Figure 3: The axonal mechanism for the generation of action potentials was modelled as a two-dimensional system, which exhibits Class I excitability. Overview of the spiking mechanism. A) Smooth transition to firing of the isolated axon under ramp-current injection (left) and the current-frequency relationship (right). Action potentials may be generated at arbitrarily low frequencies, which is characteristic of Class I excitable systems. B) Instantaneous and steady-state I-V curves, under hypothetical voltage-clamp conditions. A region of negative resistance on the instantaneous I-V curve is responsible for the self-amplifying, upward phase of the action potential. C) The phase space of the spiking system. Typically, the transition to firing occurs via a saddle node on invariant cycle bifurcation and demonstrates a clearly defined threshold around -55mV.
Figure 4: Auto-synaptic excitation may be the mechanism that underlies the characteristic plateauing activity of the N1M interneurons. A) Intracellular recording from an isolated N1M cell (Straub et al., 2002). Constant current injection triggers the generation of plateau potentials, which may be terminated by brief inhibitory (glutamate) pulses. Each plateau potential begins as a train of spikes, which gradually increase in frequency and decrease in amplitude. At the steady-state, the plateau consists of a sustained membrane depolarization, upon which truncated action potentials are superimposed. B) Simulation of the N1M model under current-clamp and brief inhibitory pulses sporadically applied. The model successfully reproduces the general characteristics of the N1M plateau. C) Schematic diagram of the hypothetical physiological mechanism, which underlies the generation of the plateau. The crucial element here is a strong ACh-dependent current, \( I_{\text{ACh}} \), which is activated by ACh released from the N1M itself and drives a self-sustained, slow-developing and long-lasting depolarization of the membrane. See main text for justification. D) The effect of the \( I_{\text{ACh}} \) reversal potential, \( E_{\text{ACh}} \), on the shape of the plateau. \( E_{\text{ACh}} \) determines the magnitude of the membrane depolarization at the fully developed plateau. E) Current-frequency relationship of the N1M model at three different values of \( E_{\text{ACh}} \). An important consequence of the design of the model is that the firing frequency is not significantly affected by the amount of injected current, but rather by \( E_{\text{ACh}} \). By determining the level of the membrane potential at the steady state, \( E_{\text{ACh}} \) controls the amount of current that flows electrotonically towards the spike-generating axonal compartment and, therefore, the firing frequency of the cell.
Figure 5: The N2v neuron exhibits conventional plateauing behaviour, which may be explained by a combination of inward and outward, slowly activated conductances. A) Intracellular recording from an N2v cell in the intact nervous system (Straub et al., 2002). The plateau consists of an underlying membrane depolarization, upon which truncated action potentials are superimposed. The membrane is spontaneously repolarized after \( \approx 1 \text{sec} \). B) Sample simulation of the N2v model under current clamp. The “two compartments” approach manages to reproduce the characteristic waveform of the N2v plateau, by assuming that the conductances for the plateau depolarization are located at the soma, while the truncated spikes on top of it are electrotonic residuals of axon-generated action potentials. C) Time evolution of a single model plateau and the underlying somatic conductances. The upwards phase of the plateau is due to the activation of a slow, inwards conductance, \( p \). The even slower activation of an outward conductance, \( q \), repolarizes the membrane and terminates the plateau. D) Behaviour of the model at three different levels of constantly injected current. For supra-threshold stimuli, the model generates regular oscillations, whose frequency increases with increasing strength of the stimulus.
Figure 6: The post-inhibitory rebound (PIR) property of the N3t may be attributed to a hyperpolarization-activated inward current, $I_h$. A) Intracellular recording of the N3t from the intact nervous system. The neuron receives consecutive inhibitory stimuli during the P and R phases of the cycle. On the rebound from the R-phase inhibition, it fires a short burst of spikes on top of a small (~5-10mV) membrane depolarization, while gradually returning to its steady state of tonic activity (Straub & Benjamin, 2001). B) Behaviour of the model, when brief inhibitory pulses are applied periodically onto the soma. The model manages to reproduce the most salient characteristics of the N3t activity. C) Time profile of a model PIR event. When the membrane potential is driven below a threshold value of -70mV for a sufficiently long time interval, the inactivation of $I_h$ is removed (i.e. the dynamic variable $q$ approaches 1). On the rebound from inhibition, $I_h$ is still de-inactivated, its total conductance is greater than 0 and a transient increase of the membrane excitability is induced. The gradual inactivation of $I_h$ at the more depolarized state drives the neuron back to its steady-state tonic activity. Notice that the decreasing part of the curve for the total conductance of $I_h$ coincides with the corresponding part of the curve for $q$ and, therefore, it has been slightly shifted to the right for convenience.
Figure 7: The SO demonstrates conventional tonic firing, under current clamp, which may be reproduced by a two-compartment model, with a passive soma and a spiking axon. A) Intracellular recording of the SO in the intact nervous system. The SO fires tonically with small size action potentials. Its electrical activity is interrupted during the R phase of the rhythm. B) Behaviour of the model SO, under constant current injection and brief, periodically applied inhibitory pulses. The soma-recorded spikes were assumed to be electrotonically attenuated action potentials generated in the axon.
Figure 8: A minimal network of reciprocally connected N1M and N2v interneurons is sufficient for generating regular oscillations, when appropriately stimulated. A) Diagram of the model network. The rhythm is typically generated by constant current injection into the N1M. B) Simulation of the network. A single feeding cycle is shaded and the most important events that determine the generation of the rhythm are also shown. (e: excitation, i: inhibition). Constant current injection into the N1M causes the neuron to fire. Through its excitatory connection onto the N2v, it slowly depolarizes the latter, until a plateau potential is triggered. The N2v activity drives the N1M below a reference value of -60mV, via a reciprocal inhibitory synapse. At so negative membrane potentials, \( I_{ACh} \) deactivates quickly and the N1M plateau is terminated. After ~1sec, the N2v terminates spontaneously, the N1M recovers, if the stimulus is still present, and a new cycle is initiated.
Figure 9: N3t provides a third phase of synaptic input to the motor neurons. A) Diagram of the model network. The N3t receives inhibitory input from the N1M and the N2v and sends inhibitory output to the N1M. B) Typical behaviour of the model network, during an N1M-driven rhythm. A single cycle is shaded and the synaptic interactions between N3t and the rest of the network are also shown. N3t receives inhibitory input in two consecutive stages, during the P and R phases of the cycle, respectively. The inhibition received from the N1M suppresses its normal tonic activity, while the inhibition received from the N2v drives the membrane potential below -70mV and triggers the de-inactivation of $I_h$. On the rebound from the R-phase inhibition, the N3t fires a burst of PIR spikes, before returning to the steady-state of tonic firing. The inhibitory input it sends to the N1M during the S phase, although not necessary for the generation of the rhythm (see Figure 8), it delays the recovery of the N1M plateau (see Figure 11). Overall, the intrinsic properties of N3t combined to the inhibitory connections of the neuron with the rest of the network are sufficient to generate a third phase of neuronal activity.
Figure 10: The N3t has a potentially modulatory role on the network, by affecting the amount of current that is needed in order to generate regular oscillations. The period of the rhythm as a function of injected current, for decreasing values of the conductance of the N3t→N1M inhibitory synapse. The inhibition that is received from the N1M during the S phase of the feeding cycle delays the onset/recovery of the N1M plateau and the initiation of the rhythm. Alternatively, for stronger S-phase inhibition, larger amounts of current are required to achieve the same frequencies.
Figure 11: The SO may drive a fast rhythm through its excitatory connections onto the N1M and N2v. A) Diagram of the model network. The SO sends excitatory input onto the N1M and N2v and receives inhibitory input from the N2v. B) A simulated fast SO-driven rhythm. A single cycle is shaded and the relevant synaptic connections are illustrated. An active SO may initiate a rhythm by inducing an N1M plateau, via its SO→N1M excitatory synapse. The SO→N2v excitatory connection provides additional input onto the N2v, thus accelerating the activation of the N2v plateau and, consequently, the termination of the N1M activity and the P phase of the cycle. As a result, SO-driven rhythms are characterized by smaller duration of the P phase.
Figure 12: The SO, but not the N1M, may drive a fast rhythm by inducing a reduction in the duration of both the P and S phases of the feeding cycle. Comparison between N1M- and SO-driven rhythms. SO-driven (A): SO-driven rhythm, whereas the SO → N2v excitatory connection has been excluded. SO-driven (B): SO-driven rhythm, whereas the SO → N2v synapse has been included. The period of the cycle as a function of injected current (top left), and the duration of each phase as a function of the total period (top right, bottom left, bottom right, respectively). When a rhythm is driven via stimulation of the N1M neuron or of the SO neuron when the SO → N2v excitation has been excluded from the network, the minimum period that may be achieved is ≈ 5-6 seconds. However, SO-driven rhythms may achieve even higher frequencies (=2-3 seconds), when the SO → N2v excitatory synapse has been included. An analysis of the duration of each phase reveals that, in the case of a full SO-driven rhythm, the duration of the P phase is, generally, shorter than that in the other two cases and reduces significantly when the period of the cycle drops below ~6 seconds. In all three cases, the duration of the P phase is constant for periods greater than ~6 seconds. Additionally, the R phase remains constant, while the S phase reduces almost linearly within the whole range of observable periods. Conclusively, SO may achieve higher-frequency rhythms that the N1M, by reducing the duration of both the P (at high frequencies) and S phases of the feeding cycle. The duration of the R phase does not contribute in the observed changes of the overall frequency of the rhythm.