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Circadian rhythmogenesis from neuromodulator- and molecular clock-mediated network mixed feedback ⋆

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Abstract: The suprachiasmatic nucleus (SCN) is a subset of neurons in charge of timekeeping circadian phenomena. As a network, it is capable of maintaining periodic activity with stable phase-locking patterns in spite of heterogeneous nodes. The mechanisms underlying SCN rhythmogenesis are still largely debated. We propose a novel biophysical SCN network model in which circadian rhythmicity emerges from the interaction of neuromodulator-mediated network positive feedback and molecular clock-mediated cellular negative feedback. Because neuromodulator action is much faster than molecular clock dynamics, our model reveals that the same "mixed" (i.e., fast positive, slow negative) feedback governing neuronal excitability also rules circadian oscillations but on several orders of magnitude slower timescales. It also reveals that, when dynamics are sufficiently slow, neuromodulators can create, instead of solely modulate, feedback loops. A mathematical abstraction of the derived SCN network makes novel predictions about the action of various SCN neuromodulators.

Keywords: Control in Neuroscience, Systems Biology, Networked Systems.

1. INTRODUCTION

The suprachiasmatic nucleus (SCN) is the master clock structure in charge of timekeeping mammal circadian phenomena (Aréchiga, 2004; Welsh et al., 2010). Although small, the SCN exhibits a complicated anatomical structure and various neuronal subtypes communicating with one another to generate robust periodic activity (Hamada et al., 2004; Maywood et al., 2006). Rhythmicity in the SCN neurons is determined internally via genetic translation-transcription feedback loops (TTFL) of well studied molecular clocks (Welsh et al., 2010), and collectively via neuromodulatory signalling (Evans, 2016). SCN neurons' electrical activity depends strongly on neuromodulators (Noguchi et al., 2017), and the loss of neuromodulatory signalling sharply diminishes rhythmic activity across the SCN (Maywood et al., 2006; Evans et al., 2013). Conversely, the loss of TTFLs, previously thought to be paramount for circadian oscillations, does not necessarily affect SCN rhythmicity (Hastings et al., 2018). Understanding SCN rhythmogenesis has thus proven a difficult task.

Many modeling efforts have been made to determine the mechanisms underlying the emergence of circadian oscillations and synchronisation in the SCN. We review two recent works that brought the molecular, electrical, and neuromodulatory level together. The model derived in (Diekman et al., 2013) originally proposed that the coupling between electrical activity and TTFLs at the single SCN neuron level is necessary for robust circadian activity. The authors in (DeWoskin et al., 2015) built upon the model of (Diekman et al., 2013) to study how network signaling modulates synchronicity in the SCN network. However, biological evidence suggests that rhythmic activity should be lost in a neuromodulator-null SCN network (Maywood et al., 2006). This observation has not been reproduced nor explained to date.

In this work, we propose a network-level extension of the model in (Diekman et al., 2013) by defining new biologically grounded dynamics for the actions of neuromodulators on cell electrical activity. The new dynamics reveal the existence of an additional neuromodulator-mediated positive feedback loop acting in parallel to the classical slower negative feedback loop of the molecular clock. The neuromodulatory feedback loop ignites and synchronizes otherwise damped and asynchronous circadian oscillations, in line with observations made at different spatial and temporal scales in molecular (Tsai et al., 2008) and excitable (Franci et al., 2018; Drion et al., 2018) dynamics. Our results suggest a novel feedback role for neuromodulators and the ubiquity of mixed feedback across timescales.

The paper is structured as follows. Section 2 presents the proposed extension for model from (Diekman et al., 2013). In Section 3 we dissect the proposed model from a feedback perspective. Numerical simulations and bifurcation analysis are presented in Section 4. Theoretical insights based on a mathematical abstraction are discussed in Section 5.

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2. A MODEL OF NEUROMODULATOR-MEDIATED NEURONAL COUPLING IN THE SCN

2.1 Single-cell modeling

We start by recalling the SCN neuron model introduced in (Diekman et al., 2013). We then extend this model to include the closed loop interaction between neuromodulators and cell excitability.

Closed-loop interactions between molecular clock and cell excitability in SCN neurons. Circadian rhythmic activity in the SCN depends both on the dynamics of gene expression (molecular clock) and cell electrical activity (excitability). In (Diekman et al., 2013), the authors propose an SCN neuron model including feedback coupling between cell excitability and the molecular clock. The electrical activity of an SCN neuron is modeled in a conductance-based framework as

$$C\dot{V} = -I_{Na} - I_K - I_{CaL} - I_{CaNL} - I_{KCa} - I_{KL} - I_{NaL},$$

where each of the ionic currents is given by

$$I_i = \overline{g}_i m_i^{a_i} h_i^{p_i} (V - E_i), \tag{1}$$

with $\dot{q} = \frac{q_\infty(V) - q}{\tau_q(V)}$, where $q_\infty(V)$ denotes the voltage-dependant steady state functions, $\tau_q(V)$ indicates the timescale in which gating variable q evolves, for $q = m_i, h_i$, and reversal potentials E_i for each ionic current i are taken from the available literature. The positive parameters $\bar{g}_i$ represent the maximal conductance of its corresponding ionic current and model how strongly the associated ion channel protein is expressed by the neuron.

The molecular clock of an SCN neuron is modeled as the negative feedback interaction of three molecular species

$$\begin{aligned} \dot{M} &= \varepsilon_{mol}((Cre)(Ebox(P^*)^n) - M), \\ \dot{P} &= \varepsilon_{mol}(M - P), \\ \dot{P^*} &= \varepsilon_{mol}(P - P^*), \end{aligned} \tag{2}$$

where $\varepsilon_{mol} = 5.6 \cdot 10^{-8}$ captures the extremely slow circadian timescale, $Ebox(P^*) = \frac{0.001}{0.001 + P^*}$ indicate enhancer-box levels, and the three variables M, P, P^* represent the mRNA levels (M) of a protein present in both its unphosphorylated (P) and phosphorylated (P^*) forms. Negative feedback oscillators are at the basis of many models of circadian rhythmicity, e.g., the Goodwin oscillator (Gonze and Ruoff, 2021).

Cell excitability and molecular clock are coupled because the cAMP-response element Cre depends on cytosolic intracellular calcium Ca_c entering the cell through calcium currents I_{CaL}, I_{CaNL} and, in return, some ion channel expressions (and therefore the associated maximal conductance $\bar{g}_i$) are affected by the concentration of the phosphorylated protein P^*. More precisely, in (Diekman et al., 2013) the authors propose that

$$Cre = Cre(Ca_c) = 10^6 Ca_c - 75,$$

with

$$\begin{aligned} \dot{Ca}_c &= -k_c(I_{CaL} + I_{CanL}) + b_c - Ca_c/\tau_c, \\ \dot{Ca}_s &= -k_s(I_{CaL} + I_{CanL}) + b_s - Ca_s/\tau_s. \end{aligned} \tag{3}$$

The model considers the effect of genetic variation over electrophysiology by redefining potassium current densities, namely

$$\overline{g}_{KCa} = \frac{a_{KCa}}{1 + \exp(R)} + b_{KCa}, \quad \overline{g}_{KL} = \frac{c_{KL}}{1 + \exp(R)}$$

with $R = 217Ebox - 0.1$.The aforementioned parameters are taken as $a_{KCa} = 198, b_{KCa} = 2, c_{KL} = 0.2$.

It is easy to see that with these modeling assumptions the coupling between cell excitability and molecular clock adds an additional negative feedback loop to the classical three-dimensional Goodwin oscillator. In (Diekman et al., 2013), this negative feedback loop is seen to *robustify* circadian oscillations and to explain key circadian excitability transitions in SCN neurons.

Closed-loop interactions between neuromodulator liberation and cell excitability in SCN neurons. Although there is no clear evidence that a neuromodulator liberated by a neuron can affect the same neuron, it is instructive to model the closed-loop interaction between neuromodulator liberation and cell excitability at the single neuron level. This preliminary step allows us to identify core mechanisms that scale up to the network level through recurrent neuromodulator-mediated coupling between different neurons.

As suggested by the evidence reported in the literature, the main player in SCN rhythmogenesis is the *vasoactive intestinal polypeptide* (VIP), followed by *arginine vasopressin peptide* (AVP) and *gastrin-releasing peptide* (GRP), while *γ-aminobutyric acid* (GABA) plays a mainly desynchronising role (Evans, 2016).

By considering the effect of neuromodulatory signaling over potassium currents (Pakhotin et al., 2006), it is possible to extend the model of Diekman et al. (2013) to include an additional feedback loop mediated by VIP (or other neuromodulators with similar modulatory effects).

Firstly, we introduce a dynamic variable x_{nmd} that lumps neuromodulator liberation from a given SCN neuron. The temporal evolution of this variable is determined by

$$\dot{x}_{\text{nmd}} = \varepsilon_{\text{nmd}}(a_{\text{nmd}} Ca_c - b_{\text{nmd}} x_{\text{nmd}}),$$

where $0 < \varepsilon_{\text{nmd}} \ll 1$ captures the time constant of peptidergic action (in simulations, we use $\varepsilon_{\text{nmd}} = 10^{-6}$), and a_{nmd} and b_{nmd} are parameters adapted from (DeWoskin et al., 2014). Biologically, the temporal evolution of x_{nmd} reflects the role of cytosolic calcium in triggering neuromodulator liberation.

A key role of neuromodulators is to affect ion channel availability. In terms of modeling this means changing modulated channels maximal conductance. If g_i is a modulated conductance, we let $g_i = g_i(x_{\text{nmd}})$ with

$$g_i(x_{\text{nmd}}) = \bar{g}_i S_{\text{nmd}}(x_{\text{nmd}})$$

where $S : (0, \infty) \to [0, 1]$ a Hill-type sigmoid. In particular, if the modeled modulator increases the maximal conductance, then $S_{\text{nmd}}(x) = H_{n,\theta}(x)$ with

$$H_{n,\theta}(x) = \frac{x^n}{\theta^n + x^n}$$

an increasing Hill-type sigmoid, whereas if the modeled modulator decreases the maximal conductance, then $S_{\text{nmd}}(x) = 1 - H_{n,\theta}(x)$.

VIP signalling mainly targets potassium channels, reducing their expression (Pakhotin et al., 2006). In our model, we include this action in the calcium activated potassium

conductance $\bar{g}_{KCa}$. In particular, extending the model in (Diekman et al., 2013), we let

$$g_{KCa} = \left(\frac{a_{KCa}}{1 + \exp(R)} + b_{KCa} \right) (1 - H_{n_{KCa},\theta_{KCa}}(x_{\text{nmd}})). \tag{4}$$

In our model, we use $a_{KCa} = 19800.0$, $b_{KCa} = 200.0$, and $n_{KCa} = 4.0$. The threshold parameter θ_{KCa} represents the strength of the neuromodulating effect over potassium currents and can be seen as a half-activation parameter for variable x_{nmd}. It serves as a tunable bifurcation parameter, and its nominal value is fixed at $\theta_{KCa} = 5.6 \cdot 10^{-6}$.

As opposed to the model of VIPergic signaling of (DeWoskin et al., 2014, 2015), in our model neuromodulatory signaling is not (or not solely) mediated by extremely slow molecular variables, which brings it back to its physiological timescale of action ranging between seconds and minutes (Pakhotin et al., 2006; Guillaumin and Burdakov, 2021).

2.2 Network-level modeling

Consider a network of N neurons modelled by (Diekman et al., 2013). Each neuron j has its own electrical $V_j, Ca_{c,j}, m_{i,j}, h_{i,j}$ and molecular M_j, P_j, P_j^* variables, and its maximal conductance parameters $\bar{g}_{i,j}$. Let $x_{\text{nmd},j}$, with

$$\dot{x}_{\text{nmd},j} = \varepsilon_{\text{nmd}}(a_{\text{nmd}} Ca_{c,j} - b_{\text{nmd}} x_{\text{nmd},j}), \tag{5}$$

model the amount of neuromodulator liberated by neuron j. Let $\bar{x}_{\text{nmd}} = (x_{\text{nmd},1}, \ldots, x_{\text{nmd},N})$. Dependence of a neuromodulated conductance $g_{i,j}$ on $\bar{x}_{\text{nmd}}$ can be modeled by generalizing single-cell modulation as

$$g_{i,j}(\bar{x}_{\text{nmd}}) = \bar{g}_i \overline{S}_{\text{nmd}}(\bar{x}_{\text{nmd}}),$$

with $\overline{S}_{\text{nmd}}(\bar{x}_{\text{nmd}}) = \overline{H}(\bar{x}_{\text{nmd}})$ or $\overline{S}_{\text{nmd}}(\bar{x}_{\text{nmd}}) = 1 - \overline{H}(\bar{x}_{\text{nmd}})$,

$$\overline{H}_{n,\theta,j}(\bar{x}) = H_{n,\theta}\left(\sum_{k=1}^{N} A_{jk} x_k \right),$$

and where $A_{jk} \geqslant 0$ is the strength of excitatory neuromodulatory projections from neuron k to neuron j. In other words, excitability properties of a given neuron are modified by directed neuromodulatory signals across the network. In the case of VIPergic coupling, we let

$$g_{KCa,j} = \left(\frac{a_{KCa}}{1 + \exp(R_j)} + b_{KCa} \right) (1 - \overline{H}_{n,\theta,j}(\bar{x}_{\text{nmd}})). \tag{6}$$

Parameters $n = n_{KCa}$ and $\theta = \theta_{KCa}$ have nominal values equal to those described in the preceding section.

Coefficients A_{jk} define a non-negative $N \times N$ weighted adjacency matrix $A = (A_{jk})$ for neuromodulatory signaling, thus indicating the existence of a neuromodulating pathway whenever $A_{jk} > 0$, and its absence otherwise. This matrix is not required to be symmetrical, homogeneous, nor having its diagonal entries equal to zero, $A_{jj} = 0$, thus allowing the existence of self-loops as well as accounting for any heterogeneity, and effectively generalising model (4).

3. NEUROMODULATOR-MEDIATED COUPLING FROM A FEEDBACK PERSPECTIVE

Despite the complexity of a network of N neurons, each modeled as the SCN neuron model of (Diekman et al., 2013) and coupled through (5), (6), it is possible to understand its emerging dynamics by studying it from a feedback perspective, both at the single node level (self-loops) and the network level. The objective in this section is to emphasize the differences in feedback structure between the original model of (Diekman et al., 2013) and the proposed extension given by (6). In particular we study the sign of the neuromodulatory feedback loops, and the importance of the timescales in which each feedback acts.

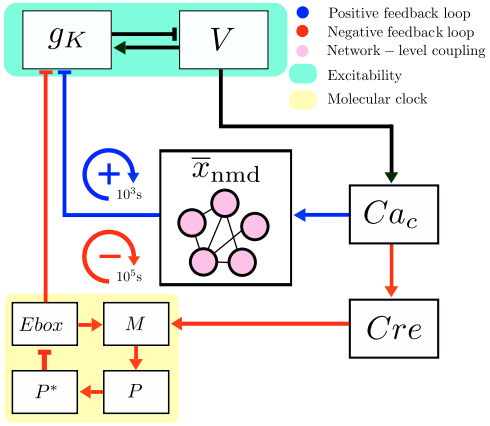

Fig. 1. Excitability in the network-level model (6) is regulated by differently signed feedback loops. The network-dependant neuromodulation pathway provides positive feedback on a faster scale, while individual molecular clocks provide negative feedback on a lower scale.

3.1 Neuromodulatory coupling through VIP provides network positive feedback

Consider a network of SCN neuron with network coupling governed by (6). As Figure 1 illustrates, there are two main feedback routes affecting the membrane potential of any given neuron: internally, from its molecular clock, and externally, from neuromodulatory signalling. The molecular route provides negative feedback, as discussed in (Diekman et al., 2013).

To compute the sign of the feedback mediated by neuromodulatory coupling, let V_j, V_k denote the membrane potential of any two neurons whose evolution is described by the SCN model of (Diekman et al., 2013) and coupled through (6). Suppose that $A_{jk} > 0$. Then the objective is to analyse the way in which changes in V_k influence the evolution of V_j. Mathematically, this reduces to studying the expression $\frac{d\dot{V}_j}{dV_k} \frac{d\dot{V}_k}{dV_j}$.

As V_k does not appear directly in the differential equation which governs V_j, it is possible to apply the Chain Rule to include all the intermediate variables between the two membrane potentials. This is done in an analogous manner to how the authors in (Franci et al., 2013) and (Drion et al., 2015) study excitability through dynamic input conductances, by computing terms of the form $\frac{\partial \dot{V}}{\partial x_i} \frac{\partial \dot{x}_i}{\partial V}$ to describe the feedback between membrane potential V and a gating variable x_i.

Membrane potentials act on each other via a calcium-regulated pathway. V_k affects V_j indirectly by influenc-

ing $Ca_{c,k}$ according to (3), which in turns modifies the amount of liberated modulator according to (5), which finally modulates the potassium conductance $g_{KCa,j}$ in $\dot{V}_j$ according to (6). The following lemma shows that the resulting interaction is excitatory.

Lemma 1. Consider system (6) and let j, k be two indices which satisfy $A_{jk} > 0$. Then

$$\frac{\partial \dot{V}_j}{\partial x_{\mathrm{nmd},k}} \frac{\partial \dot{x}_{\mathrm{nmd},k}}{\partial Ca_{c,k}} \frac{\partial \dot{Ca}_{c,k}}{\partial V_k} > 0.$$

Proof. We start by studying the far-right differential term. Recall that, according to (3), $\dot{Ca}_{c,k} = -k_c I_{Ca,k} + b_c - Ca_{c,k}/\tau_c$, where $I_{Ca,k} = I_{CaL,k} + I_{CanL,k}$ denotes the inward, voltage-activated total calcium current for the kth neuron. Using (1) and recalling that calcium of currents depolarize the neuron as the membrane potential is increased, it follows that $\frac{\partial I_{Ca,k}}{\partial V_k} < 0$, which implies $\frac{\partial \dot{Ca}_{c,k}}{\partial V_k} = -k_c \frac{\partial I_{Ca,k}}{\partial V_k} > 0$. The neuromodulator dynamics (5) yield $\frac{\partial \dot{x}_{\mathrm{nmd},k}}{\partial Ca_{c,k}} = \varepsilon_{\mathrm{nmd}} a_{\mathrm{nmd}} > 0$. Finally, since the molecular term in $g_{KCa,j}$ remains a positive constant, differentiating membrane potential evolution $\dot{V}_j$ with respect to neuromodulator $x_{\mathrm{nmd},k}$ is essentially reduced to computing the derivative $-\frac{\partial}{\partial x_{\mathrm{nmd},k}} \left(1 - H_{n_{KCa},\theta_{KCa}} \left(\sum_{l=1}^{N} A_{jl} x_{\mathrm{nmd},l}\right)\right)$ $= (-A_{jk}) \left(-H'_{n_{KCa},\theta_{KCa}} \left(\sum_{l=1}^{N} A_{jl} x_{\mathrm{nmd},l}\right)\right)$. Since $A_{jk} > 0$ by hypothesis and $H_{n,\theta}$ are defined as strictly increasing sigmoid functions, we conclude $\frac{\partial \dot{V}_j}{\partial x_{\mathrm{nmd},k}} > 0$, whence the result follows. □

Lemma 1 implies that all network feedback mediated by neuromodulatory loops is necessarily positive. Indeed, by assuming there exists a closed-loop path in the network from neuron j to itself, *i.e.*, there exist $k_1, \ldots, k_p$ such that $A_{jk_1} A_{k_1 k_2} \cdots A_{k_{p-1} k_p} A_{k_p j} > 0$, Lemma 1 concludes

$$\frac{\partial \dot{V}_j}{\partial V_{k_1}} \frac{\partial \dot{V}_{k_1}}{\partial V_{k_2}} \cdots \frac{\partial \dot{V}_{k_{p-1}}}{\partial V_{k_p}} \frac{\partial \dot{V}_{k_p}}{\partial V_j} > 0.$$

3.2 Mixed feedback in circadian oscillations

Modeling circadian phenomena involves variables with timescales ranging from the order of milliseconds (electrophysiology) to the order of hours and days (genetic). In the case of the original (Diekman et al., 2013) model, as well as the proposed extension based on (6), the circadian timescale is the slowest one. Genetic rhythms exhibit significant changes only over the course of several hours, and thus the negative feedback of the molecular clock influences the system on a timescale that is eight orders of magnitude slower that then the electrophysiological timescale, as modeled by $\varepsilon_{\mathrm{mol}} = 5.6 \cdot 10^{-8}$ in (2). On the other hand, neuromodulation is significantly faster, with noticeable changes in neuromodulator levels being observable in the order of seconds to minutes (Guillaumin and Burdakov, 2021). In the case of the SCN model of (Diekman et al., 2013) augmented with (6), this is done by setting $\varepsilon_{\mathrm{nmd}} = 10^{-6}$, which is two orders of magnitude faster than genetic dynamics.

In other words, the positive feedback mediated by network-level neuromodulation acts on a much faster timescale than the negative feedback mediated by the genetic dynamics of the molecular clock. This defines a "mixed" feedback motif which has been shown as a key concept in understanding robustness and modulation of single neuron and neuronal network excitable dynamics (Franci et al., 2018; Drion et al., 2019, 2018). Here, we propose that the same feedback principle governs robust and tunable circadian oscillations.

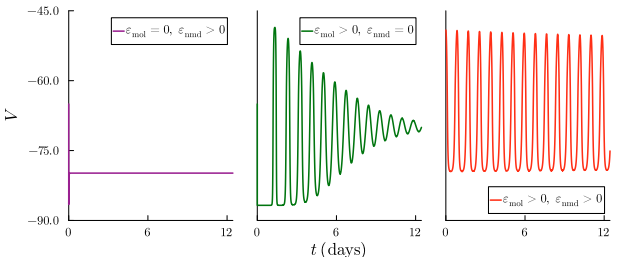

Fig. 2. Feedback through neuromodulation and gene expression are both necessary for sustained circadian activity at the single cell level. In single-cell model (4), blocking either gene expression (*left*, $\varepsilon_{\mathrm{mol}} = 0$) or self-loop neuromodulation (*center*, $\varepsilon_{\mathrm{nmd}} = 0$) results in silent or damped electrical behavior.

4. SIMULATIONS AND BIFURCATION ANALYSIS

We now present numerical simulations of the single-cell and network models to explore the role of molecular and neuromodulatory feedback loops in creating circadian oscillations.

Remark: because in our model electrophysiological gating variables of are eight orders of magnitude faster than genetic variables, we approximate the former as instantaneous, which leads to a $7N$-dimesional system of ODEs for N simulated neurons.

4.1 Circadian clock-mediated negative feedback cooperates with neuromodulator-mediated positive feedback to ignite robust circadian oscillations

Figure 2 shows that blocking either molecular clock-mediated negative feedback (left) or neuromodulator-mediated positive feedback (center) at the single cell level in the model of (Diekman et al., 2013) augmented with (4) stops circadian oscillations. The key role of molecular negative feedback for circadian oscillations was already highlighted in (Diekman et al., 2013). Here we extend this observation by revealing that neuromodulator positive feedback is also key to circadian rhythmicity at the single cell level.

Figure 3 generalizes the observations made in Figure 2 to the network level by analyzing the behavior of the model of (Diekman et al., 2013) augmented with (6). In the absence of molecular clock-mediated negative feedback, the oscillators stabilize rapidly at a low potential. In the absence of network-level neuromodulator-mediated positive feedback the oscillators are damped, converging to a low potential as well. Switching on both feedback loops leads to sustained synchronous oscillations as revealed by plotting the total-variation synchronicity measure

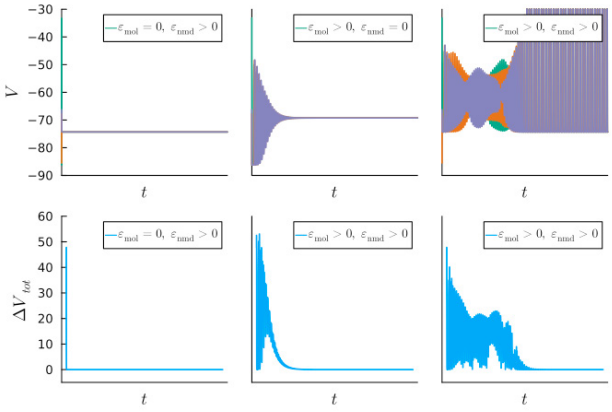

Fig. 3. Feedback through neuromodulation and gene expression are both necessary for synchronous circadian activity at the network level. *Top:* network membrane potential evolution in model. Timescale parameters are the same as in Figure 2. *Bottom:* Total variation measure of synchronicity for each parameter scheme. This measure tends to zero for nominal timescale values, corresponding to perfect synchronisation. $N = 3$ neurons, $T = 72$ days.

$$\Delta V_{tot} = \sqrt{\sum_{j} \sum_{k>j} (V_j - V_k)^2}.$$

At the network level, the positive feedback provided by neuromodulatory coupling has a dual role: it ignites sustained oscillations and it synchronizes the oscillators. This in line both with biological observations (Maywood et al., 2006) as well as theoretical results derived in (Juarez-Alvarez and Franci, 2021).

4.2 Positive feedback strength controls oscillation existence and amplitude through a Hopf bifurcation.

To explore in more detail the mechanisms through which neuromodulator-mediated positive feedback ignites oscillations, we present a detailed numerical bifurcation analysis of the single cell model. An analogous analysis for the network model would be computationally intense and is left for future work, but preliminary explorations suggest the bifurcation mechanisms at work are the same as the single cell level.

The threshold, or half-activation, parameter θ_{KCa} appearing in (4), (6) determines the range in which the sigmoid S_{nmd} is steeper, that is, the range in which the neuromodulator-mediated positive feedback is the strongest. When θ_{KCa} is large enough, positive feedback is close to zero in the physiological range of variables, because the sigmoid S_{nmd} is close to flat in that range. Therefore, it must be possible to observe a transition from damped to sustained oscillations by decreasing θ_{KCa} and for all other parameters as in Figure 2 (right).

Figure 4 computationally confirms this intuition. For θ_{KCa} above a critical value $\theta^*_{KCa} \approx 7.67 \cdot 10^{-6}$ the only model equilibrium is locally exponentially asymptotically stable. For $\theta_{KCa} = \theta^*_{KCa}$ the equilibrium possesses a pair of conjugate purely imaginary eigenvalues. Finally, the same

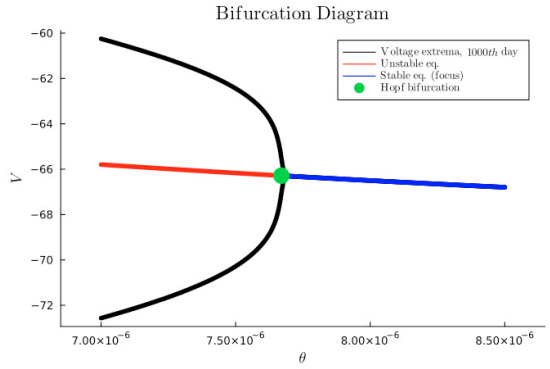

Fig. 4. Varying the half activation parameter θ_{KCa} shifts neural activity from sustained to damped oscillations through a supercritical Hopf bifurcation. As θ_{KCa} is varied, the stability of a bifurcating equilibrium point is determined by numerically computing the spectrum of the system's jacobian matrix.

equilibrium becomes unstable for $\theta_{KCa} < \theta^*_{KCa}$. This transition corresponds to a Hopf bifurcation.

To explore the criticality of the detected Hopf bifurcation we run very long (1000 days) simulations and plot values of the extrema of $V(t)$ during the last day of simulation. The amplitude of the stable limit cycle surrounding the unstable equilibrium decreases to zero as θ_{KCa} approaches θ^*_{KCa} and there are no detectable stable limit cycles for $\theta_{KCa} > \theta^*_{KCa}$, which indicates that the Hopf bifurcation is supercritical.

We conjecture that if the neuromodulatory coupling matrix A is irreducible, that is, it defines a strongly connected graph, than the network-level model is also organized by a supercritical Hopf bifurcation. This intuition is motivated by the single-cell results of this section and by the results derived in (Juarez-Alvarez and Franci, 2021), which we recall in the following section.

5. MIXED-FEEDBACK OSCILLATOR VIEWPOINT ON CIRCADIAN RHYTHMOGENESIS

5.1 A mathematical abstraction

The simple model of coupled slow-fast oscillators studied in (Juarez-Alvarez and Franci, 2021) possesses the same mixed-feedback structure as the biophysical model presented in this paper. Given N slow-fast oscillators with state space $\{(x_j, y_j)\}_{j=1}^N$, the model is defined as the system of $2N$ ordinary differential equations

$$\begin{aligned} \dot{x}_j &= -x_j - y_j + S\left(\alpha x_j + \sum_{k=1}^{N} A^e_{jk} x_k\right), \\ \dot{y}_j &= \varepsilon(x_j - y_j), \end{aligned} \tag{7}$$

for every $j \in \{1, \dots, N\}$. In the context of model (7), $S : \mathbb{R} \to \mathbb{R}$ is a strictly increasing sigmoid function which satisfies $S(0) = 0$ and $S'(0) > 0$. Parameter $\varepsilon \in (0, 1)$ establishes a timescale for the evolution of slow components y_j, and parameter $\alpha \geqslant 0$ determines intrinsic oscillations. The coupling matrix A^e is a assumed to be non-negative.

The fast variables x_j are mathematical abstractions of the electrophysiological and neuromodulatory variables of the biophysical model studied in this paper. The slow variables y_j are mathematical abstractions of the molecular clock variables. As in the biophysical models, it is easy to see that fast variables are coupled through positive feedback loops while slow variables provide individual negative feedback.

It was shown in (Juarez-Alvarez and Franci, 2021) that if $A^e = \beta A$ where A is a non-negative, irreducible matrix and $\beta > 0$, then model (7) undergoes a supercritical Hopf bifurcation as β increases. The case for $N = 1$ slow-fast oscillators in (Juarez-Alvarez and Franci, 2021) behaves similarly to the unicellular system depicted in Figure 4, going through a supercritical Hopf bifurcation as intrisic parameter α is increased. Taking this into account, model (7) and the biophysical system of N SCN neurons coupled through (6) are expected to possess the same mixed-feedback structure and be organized by the same bifurcations, which would make (7) a valid qualitative model to study circadian rhythmogenesis from a mathematical perspective.

5.2 Biological predictions

A key prediction of model (7) is that if neuromodulatory coupling is fully excitatory in the sense of Lemma 1, then all neurons of the SCN should oscillate in phase, which is in contrast with biological observations (Inouye and Shibata, 1994; Ono et al., 2021). VIP provides excitatory coupling between SCN neurons but other neuromodulators are present in the SCN. Of these, AVP and GRP are known to be widespread in SCN signaling but their action on cellular ionic currents has not yet been studied in detail. Our prediction is that either or both must provide inhibitory coupling between SCN neurons, in the sense that they either upregulate inhibitory currents or downregulate excitatory ones.

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