Modeling of Oscillations of Endocrine Networks with Feedback.

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General principles in endocrine network modeling

Numerous studies document that hormone delivery pattern to target organs is crucial to the effectiveness of their action. Hormone release could be altered by pathophysiology and differences in endocrine output mediate important intraspecies distinctions, like for example, some of the sexual dimorphism in body growth and gene expression in humans and rodents. Accordingly, the mechanisms controlling the dynamics of various hormones had become lately the object of extensive biomedical research. Intuitive reconstruction of endocrine axes is challenged by their high complexity, due to multiple intervening time-delayed nonlinear feedback and feedforward inputs from various hormones and/or neuroregulators. Consequently, quantitative methods have been developed, to complement qualitative analysis and laboratory experiments and reveal the specifics of hormone release control. The emerging mathematical models interpret endocrine networks as dynamic systems and attempt to simulate and explain their temporal behavior.[1-6]

This chapter focuses on the mathematical approximation of endocrine oscillations in the framework of a modeling process structured in three formal phases:

(a) Data analysis (examining the available data). We start with studying the available observations and experimental results, by examining the hormone time series and determining the specifics of the observed profiles. This might include pulse detection, analysis of the variability and orderliness, verifying the baseline secretion and half-life, detecting the frequency of the oscillations. We identify those phenomena that should be explained by the modeling effort, for example, some specific property of the hormone profiles, combined with selected feedback experiments.

(b) Qualitative analysis (designing the formal network). This stage uses the information, collected in phase (a) and outlines an intuitive functional scheme of the systems underlying physiology. Qualitative analysis of the available data[7] identifies the key elements and their interaction and organizes them as a set of nodes and conduits in a *formal endocrine network*. The *main hypothesis* states that this formal network explains the selected in phase (a) specifics in the experimental data.

(c) Quantitative analysis (dynamic modeling). At this phase the endocrine network is interpreted as a dynamic system and described with a set of

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[1] Farhy LS, Straume M, Johnson ML, Kovatchev BP, and Veldhuis JD. *Am J Physiol Reg Integr Comp Physiol*, 281: R38-R51, 2001.

[2] Farhy LS, Straume M, Johnson ML, Kovatchev BP, and Veldhuis JD. Unequal autonegative feedback by GH models the sexual dimorphism in GH secretory dynamics. *Am J Physiol Reg Integr Comp Physiol*, 282: R753-R764, 2002.

[3] D.M. Keenan and J. D. Veldhuis, Am. J. Physiol. **281**, R1917(2001).

[4] D.M. Keenan and J. D. Veldhuis, Am. J. Physiol. **280**, R1755(2001).

[5] L. Chen, J.D. Veldhuis, M.L. Johnson, and M. Straume. In: *Methods in Neurosciences.* New York: Academic Press, 270 (1995).

[6] C. Wagner, S.R. Caplan, and G.S. Tannenbaum, *Am. J. Physiol.* **275**, E1046 (1998).

[7] O. Friesen and G. Block, Am. J. Physiol. **246**, R847 (1984).

coupled ordinary differential equations (ODE). They give the time derivative of each network node and approximate all system positive and negative dose-responsive control loops. The parameters in the ODEs must have a clear physiological meaning and are determined by comparing the model output with the available data (phase (a)) as we attempt to address the main hypothesis (phase (b).

The outcome of the modeling effort is a *conditional* answer to the main hypothesis. It formulates necessary physiological assumptions (additional to the main hypothesis) that would allow the formal network to explain the observed data specifics. This further refines the hypothesis and generates new questions to be addressed experimentally.

The general modeling scheme, anticipates that the qualitative analysis of the hormone secretion dynamics outlines the formal endocrine network by determining its nodes and conduits. As discussed in[7] the main source of oscillations in biology are feedback loops with delay. However, not every network with feedback generates periodic behavior[8]. The main goal of this work is to illustrate via series of abstract examples different conditions, under which oscillations can emerge. To this end, we perform quantitative analysis on various abstract endocrine networks, interpreted as dynamic systems. Thus, we will be mainly concerned with phase (c) (above) and its relations to phases (a) and (b).

We start by describing the approximation of the basic element of an endocrine network: the dynamics of the concentration of a single hormone, eventually controlled by one or more other regulators (system nodes). Further, this is used in the simulation and analysis of different feedback networks. The main concepts are illustrated on abstract 2-node/1-feedback reference models. System parameters are introduced on the basis of their physiological meaning and the effect of their modification is examined. Oscillations due to perturbations of systems with damped periodicity are distinguished from oscillations of systems with a true periodic solution (limit cycle). Additionally, we simulate basic laboratory experimental techniques, discuss some of their limitations, and suggest alternatives to reveal more network details.

It should be noted that the theory behind most of the examples in this chapter is not trivial. This is especially valid for those models that include one or more direct delays in the core system. We avoid the abstract mathematical details to make the presentation accessible to a variety of bio-scientists. The simulated networks are abstract and do not correspond to a particular endocrine system. However, the constructs and the modeling techniques can be easily adapted to fit a particular physiology.

Simulating the concentration dynamics of a single hormone

In this section we describe the quantitative approximation of the concentration dynamics of a single hormone in the abstract pool, where it is secreted (not

[8] R. Thomas, R. D'Ari, and N. Thomas, "Biological feedback", CRC Press, 1990.

synthesized). As described elsewhere (see for example, [9]), we assume that the hormone concentration rate of change in a certain pool depends on two processes - secretion and ongoing elimination. The quantitative description is given by the ordinary differential equation

$$(1) \qquad \frac{dC}{dt} = -\alpha C(t) + S(t).$$

Here, $C(t)$ is the hormone concentration in the corresponding pool, t is the time, $S(t)$ is the rate of secretion and the elimination is supposed to be proportional to the concentration.

Deconvolution technique, employed to describe hormone pulsatility[9], can be used as an alternative approach to introducing Eq. (1). In this context, the observed hormone concentration is described by a convolution integral

$$(2) \qquad C(t) = \int_0^t S(\tau)E(t-\tau)d\tau$$

where S is a secretion function, and E describes the removal of the hormone from the pool. For the purposes of this presentation, E is required to correspond to a model with one half-life. In particular, we assume that the elimination function $E(t)$ satisfies the initial value problem

$$(3) \qquad \begin{aligned} &\frac{dE(t)}{dt} = -\alpha E(t) \\ &E(0) = 1 \end{aligned}$$

with some rate of elimination $\alpha > 0$. Consequently, it is easy to see that Eqs. (2) and (3) imply that the right-hand side of Eq. (1) describes the rate of change of $C(t)$. And since the solution of Eq. (3) is the function $E(t) = e^{-\alpha t}$ the hormone concentration (the solution of Eq. (1)) is described as the convolution integral

$$C(t) = \int_0^t S(\tau)e^{-\alpha(t-\tau)}d\tau$$

Now, suppose that the secretion rate $S = S_A$ (of a hormone A) do not depend explicitly on t and is controlled by some other hormone B. We write $S_A = S_A(C_B(t))$, where $C_B(t)$ is the concentration of B. In the sequel, S_A is called a control function and its choice, albeit arbitrary to some extent, should conform to a set of general rules.

(a) Minimal and maximal exogenous levels

Denote by $C_{A,\min}$ and by $C_{A,\max}$ the minimal and maximal values (experimentally established or hypothetical) for the concentration of hormone A. Typically (but not always), $C_{A,\min}$ is associated with the baseline secretion and $C_{A,\max}$ corresponds to the maximal attainable concentration of exogenous A (on variety of conditions, including responses to external sub-maximal stimulation). Accordingly, the control function S_A must satisfy the inequalities:

[9] J.D. Veldhuis and M.L. Johnson, *Methods Enzymol*. **210**, 539 (1992)

$$C_{A,\min}/\alpha \le \min(S_A) \le \max(S_A) \le C_{A,\max}/\alpha$$

(b) Monotonous and nonnegative

The control function must be nonnegative, since the secretion rate is always nonnegative, and monotone (with some rare exceptions briefly mentioned in the sequel). It will be monotone increasing if represents a positive control. If the control is negative, it will be decreasing.

There are many ways to introduce a control function in an acceptable mathematical form. As many authors do, we use nonlinear, sigmoid functions, known as up- and down-regulatory Hill functions (see [8] for details)

$$(4) \qquad F_{up(down)}(G) = \begin{cases} \dfrac{[G/T]^n}{[G/T]^n + 1} & (up)\ or \\ \dfrac{1}{[G/T]^n + 1} & (down) \end{cases}$$

where $T > 0$ is called a threshold and $n \ge 1$ is called a Hill coefficient. It should be noted that $F_{up} = 1 - F_{down}$ and $F_{up(down)}(T) = 1/2$. These functions are exemplified in the plots in Fig. 1 (for $n = 5$ and $T = 50$).

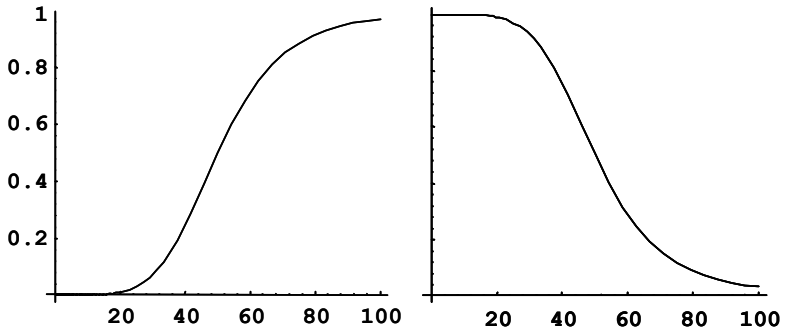

Fig. 1. Exemplary profiles of up-regulatory (left panel) and down-regulatory (right panel) Hill functions. In both examples $n = 5$ and $T = 50$.

They are monotone and map $F : (0,\infty) \to (0,\infty)$; the Hill coefficient n controls the slope (which also depends on T), and the inflection point I_F is given by:

$$I_F = T\left(\frac{n-1}{n+1}\right)^{\frac{1}{n}} \text{ for } n \ge 2$$

When $n = 1$ (Michaelis-Menten type equation) the function has no inflection point and its profile is a branch of a hyperbola. If n is large (values, as large as 100, exist in biology [10, 11]) the control function acts almost as an on/off switch.

Using Hill functions, we write the term controlling the secretion of A in the form:

[10] P.V. Vrzheshch, O.V. Demina, S.I. Shram, and S.D. Varfolomeev. *FESB Letters,* **351**(2), 168 (1994).
[11] T. Mikawa, R. Masui, and S. Kuramitsu. *J Biochem* **123**(3), 450 (1998).

$$(5) \qquad S_A(C_B) = aF_{up,(down)}(C_B) + S_{A,basal},$$

where $S_{A,basal} \geq 0$ is independent of B and controls the basal secretion of A. The quantities $(a + S_{A,basal})/\alpha$ and $S_{A,basal}/\alpha$ represent the above mentioned $C_{A,\max}$ and $C_{A,\min}$, respectively.

As mentioned earlier, on certain occasions, the monotonousness of the control function may be violated. For example, if might happen that at low to medium concentrations a substance is a stimulator, while at high concentrations it is an inhibitor. Thus, the control function is non-monotonous and can be written as a combination of Hill functions[8]:

$$S_A(G) = a\frac{[G/T_1]^{n_1}}{[G/T_1]^{n_1}+1}\frac{1}{[G/T_2]^{n_2}+1}, \quad T_1 < T_2.$$

Next, assume that instead of one, two hormones control the secretion of A. We denote them by B and C with corresponding concentrations $C_B(t)$ and $C_C(t)$. The control function $S_A = S_A(C_B, C_C)$ depends on the specific interaction between A from one side, and B and C from another[8]. For example, if both B and C stimulate the secretion of A

$$(6) \qquad S_A(C_B, C_C) = a_B F_{up}(C_B) + a_C F_{up}(C_C) + S_{A,basal}$$

if B and C act independently, or

$$(7) \qquad S_A(C_B, C_C) = aF_{up}(C_B)F_{up}(C_C) + S_{A,basal}$$

if B and C act simultaneously (the secretion of A requires the presence of both). On the other side, if for example, the secretion of A is stimulated by B, but suppressed by C, the control function can be introduced as

$$(8) \qquad S_A(C_B, C_C) = aF_{up}(C_B)F_{down}(C_C) + S_{A,basal},$$

or

$$(9) \qquad S_A(C_B, C_C) = a_B F_{up}(C_B) + a_C F_{down}(C_C) + S_{A,basal}.$$

Note, that Eq. (8) simulates a non-competitive and simultaneous action of B and C. If B and C compete as they control the secretion of A, the secretion term can be described with a modified Hill function:

$$(10) \qquad S_A(C_B, C_C) = a\frac{(C_B/T_B)^{n_B}}{(C_B/T_B)^{n_B} + (C_C/T_C)^{n_C} + 1} + S_{A,basal}.$$

Oscillations driven by a single system feedback loop

In this section we discuss in detail networks with a single (delayed) feedback loop that can generate oscillatory behavior. We focus on 2-node/1-feedback networks, in which the concentration of one hormone A regulates the secretion of another hormone B, which in turn controls the release of A. This construct can generate oscillations, even if there is no explicit (direct) delay in the feedback[12]. However, in this case the oscillations will fade to the steady state of the system. A non-zero delay and a large nonlinearity in the control functions (sufficiently high Hill coefficients) guarantee steady periodic behavior, due to the

[12] The thresholds in the control functions provide implicit delays in the corresponding conduits.

existence of a non-trivial limit cycle. On the other hand, a network may incorporate a single feedback loop by means of only one or more than two nodes. We comment on some peculiarities of such models in the last section.

FORMAL 2-NODE/1-FEEDBACK NETWORK. We study the abstract endocrine networks shown on Fig. 2.

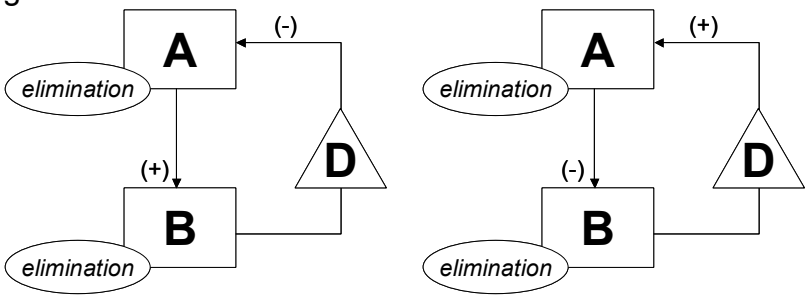

Fig. 2. Formal network of a two-node/one-feedback oscillator. The left panel depicts a network in which the main hormone B is stimulated, while the scheme on the right shows a model in which B is inhibited. D denotes a delay in the interconnection. In both networks A and B are subject to elimination.

These particular examples anticipate that two hormones, A and B are continuously secreted (driven by nonrhythmic excitatory input) in certain pool(s) (systemic circulation, portal blood, etc.), where they are subject to elimination. The release of hormone B is up-(down-)regulated by hormone A. Hormone B itself, exerts a negative (positive) delayed feedback on the secretion of A. The A/B interactions are assumed to be dose-responsive. The resulting delayed control loop is capable of driving hormone oscillations, if certain conditions (discussed below) are provided.

To formalize the networks depicted in Fig. 2, we denote the concentrations of hormones A and B by $C_A(t)$ and $C_B(t)$, respectively. We assume that the elimination of each hormone is proportional to its concentration with positive constants α and β. The secretion rate S_A of A is supposed to depend on the history of the concentration of B and *vice versa*. In particular, we assume that $S_A(t) = S_A(h_1[C_B(t)])$ and $S_B(t) = S_B(h_2[C_A(t)])$. The functional h_1 (h_2) incorporates the lag in the action of B on A (A on B). To formally introduce the delays, one can account for the time-averaged effect of the hormone action in a past time interval related to the current moment[4]. However, this method requires two parameters for each delayed action – the onset and the termination of the delayed action (see[4] for details). Here, in order to keep the model as minimal as possible, we use a "direct" delay (with only one parameter for each delayed control action) and assume that the secretion control functions can be written as

$$S_A(t) = S_A(C_B(t - D_B)) \text{ and } S_B(t) = S_B(C_A(t - D_A)),$$

with some non-negative delay times D_A and D_B. Then, the system of ordinary (non-linear) delayed differential equations, which describes a formal two-node/one-feedback endocrine network (Fig. 2), has the form

$$(11)\qquad \begin{aligned} \frac{dC_A}{dt} &= -\alpha C_A(t) + S_A(C_B(t-D_B)) \\ \frac{dC_B}{dt} &= -\beta C_B(t) + S_B(C_A(t-D_A)) \end{aligned}$$

with some elimination constants $\alpha, \beta > 0$, lag times $D_A, D_B \geq 0$, and secretion rate control functions $S_A, S_B \geq 0$.

REFERENCE SYSTEMS. To describe the dose-responsive relationships between A and B, corresponding to the network from Fig. 2, left panel, we use the recommendations outlined in "Hormone release approximation" (Eq. (5)). We write the control functions that appear in (11) as follows

$$S_A(C_B(t-D_B)) = aF_{down}(C_B(t-D_B)) + S_{A,basal}$$
$$S_B(C_A(t-D_A)) = bF_{up}(C_A(t-D_A)) + S_{B,basal}$$

With this special choice, the core system of first order non-linear differential equations, describing the network from Fig. 2 (left), have the form:

$$(12)\qquad \begin{aligned} \frac{dC_A}{dt} &= -\alpha C_A(t) + S_{A,basal} + a\frac{1}{(C_B(t-D_B)/T_B)^{n_B}+1} \\ \frac{dC_B}{dt} &= -\beta C_B(t) + S_{B,basal} + b\frac{(C_A(t-D_A)/T_A)^{n_A}}{(C_A(t-D_A)/T_A)^{n_A}+1} \end{aligned}$$

The units in this model are

$C_A,\ C_B,\ T_A, T_B$	mass/volume
$a, b, S_{A,basal}, S_{B,basal}$	mass/volume/time
α, β	time^{-1}
D_A, D_B	time

However, in the sequel we avoid specifying the specific unit and the simulated profiles have arbitrary magnitude, which could be rescaled with ease to fit a desired physiology.

In most of the simulations we assume no basal secretions and a direct action of A on B (no delay). This transforms the core equations (Eq. (12)) into:

$$(13)\qquad \begin{aligned} \frac{dC_A}{dt} &= -\alpha C_A(t) + a\frac{1}{(C_B(t-D_B)/T_B)^{n_B}+1} \\ \frac{dC_B}{dt} &= -\beta C_B(t) + b\frac{(C_A(t)/T_A)^{n_A}}{(C_A(t)/T_A)^{n_A}+1} \end{aligned}$$

Note, that solving these equations for $t \geq t_0$ requires the initial condition for C_B to be given on the entire interval $[t_0 - D_B, t_0]$.

From the special form of Eq. (13) we could easily derive that after some time (depending on the initial conditions), the solutions will be bounded away from zero and from above. More formally, for any $\varepsilon > 0$ (and we may choose ε as

small as we like), there exists $t_0 > 0$ (depending on ε, the initial conditions and the system parameters), such the for $t > t_0$ the following inequalities hold and provide upper and lower bounds on the solution of Eq. (13):

$$(14)\qquad \begin{aligned} 0 < \frac{a}{\alpha}\frac{1}{\left(\frac{b}{\beta T_B}\right)^{n_B}+1} - \varepsilon \le C_A(t) \le \frac{a}{\alpha} + \varepsilon \\ 0 < \frac{b}{\beta}\frac{1}{\left(\frac{T_A}{\min C_A}\right)^{n_A}+1} - \varepsilon \le C_B(t) \le b/\beta + \varepsilon \end{aligned}$$

The upper bounds above are absolute system limits. For example, the model response to exogenous A-bolus cannot exceed the value b/β. However, since $C_A < a/\alpha$, we get from Eq. (14) that the actual endogenous peak concentration of B will never reach b/β. In fact, if there is no external input of energy in the system, it will be less than

$$(15)\qquad C_B(t) \le \frac{b}{\beta}\frac{1}{\left(\frac{\alpha T_A}{a}\right)^{n_A}+1} < \frac{b}{\beta}.$$

Hence, changes in four parameters (a, α, n_A, T_A) can model a difference between the maximal amplitude of the internally generated peaks and the eventual response to external stimulation. All estimates may be refined through a recurrent procedure inherent in the core system (Eq. (13)). For example, one can combine the two inequalities Eq. (14) to get an explicit lower bound for C_B:

$$(16)\qquad \frac{b}{\beta}\frac{1}{\left(\frac{\alpha t_A\left(\left(\frac{b}{\beta T_B}\right)^{n_B}+1\right)}{a}\right)^{n_A}+1} \le C_B(t)$$

Accordingly, we can use this to write an explicit upper bound for C_A:

$$C_A \le \frac{a}{\alpha}\frac{1}{\left(\frac{C_{B,\min}}{T_B}\right)^{n_B}+1} \le \frac{a}{\alpha}\frac{1}{\left(\frac{M}{T_B}\right)^{n_B}+1}, \text{ where } M = \frac{b}{\beta}\frac{1}{\left(\frac{\alpha T_A\left(\left(\frac{b}{\beta T_B}\right)^{n_B}+1\right)}{a}\right)^{n_A}+1}$$

These inequalities can help to determine reasonable values for the model parameters.

It is easy to see that (since the control functions are monotonously decreasing and increasing) the system Eq. (13) has a unique fixed point (steady state). It can be shown that if there is no delay ($D_A = D_B = 0$) the fixed point is asymptotically stable (a node or a focus) and attracts all trajectories in the phase space: Fig. 3, panel A. However, even a single non-zero delay (as in Eq. (13)) might change the properties of the steady state. The particular stability analysis is non-trivial, and consists of investigating the real part of eigenvalues, which are roots of equation containing a transcendental term, involving the delay. In the examples that follow, we will encounter one of the two situations depicted in Fig. 3: the steady state will be either an attractor (panel A) or a repellor (panel B), and in the latter case, there will exist a unique asymptotically stable periodic solution (which encircles the fixed point in the phase space) acting as a global limit cycle by attracting all trajectories (except the one originating from the fixed point).

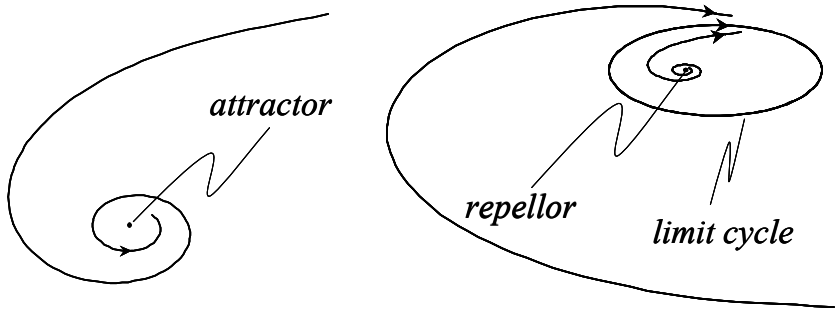

Fig. 3. Illustrative trajectories in the space (C_A, C_B) if the steady state is an attractor (panel A) or a repellor (panel B). In the latter case, a unique asymptotically stable periodic solution acts as a limit cycle and attracts all other trajectories (except the fixed point).

OSCILLATIONS GENERATED BY A PERIODIC SOLUTION. In this section we present two specific examples describing the networks in Fig. 2. The core system of delayed ODE for the reference models will have unique periodic solution and unique repelling fixed point (Fig. 3, B).

Let consider a construct, described by the following core equations:

$$\begin{aligned} \frac{dC_A}{dt} &= -1C_A(t) + 5\frac{1}{[C_B(t-3)/20]^2 + 1} \\ \frac{dC_B}{dt} &= -2C_B(t) + 500\frac{[C_A(t)/5]^2}{[C_A(t)/5]^2 + 1} \end{aligned} \tag{17}$$

These equations simulate the network shown in Fig. 2, left panel (A is a stimulator). The parameters were chosen to guarantee stable oscillations: Fig. 4.

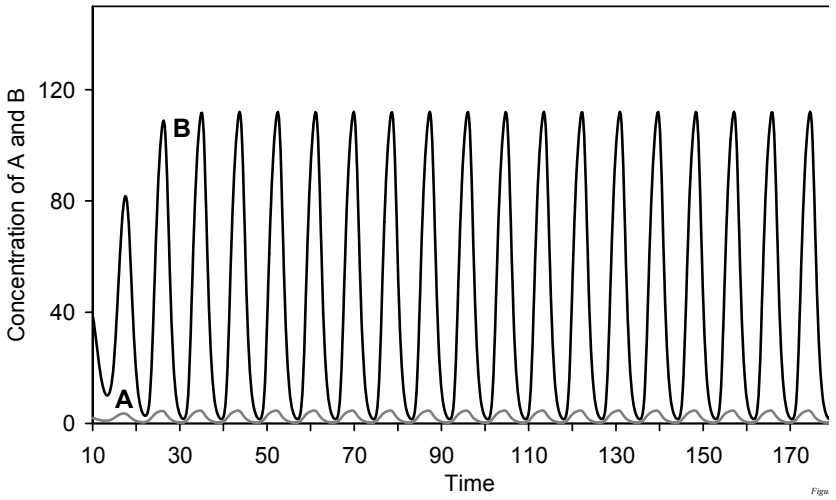

Fig. 4 Dynamics of the concentration of A (the lower profile) and B, for the reference model described by Eq. (17).

Later, we show how the parameter choice affects the periodicity.

Even in this simple example, we have a variety of possibilities to model the specific interactions between A and B. In the above example we have surmised:

(a) The maximal attainable amplitude of C_B is 250.

(b) The maximal attainable amplitude of C_A is 5.

(c) The threshold t_A is higher then the endogenous levels of C_A.

(d) The threshold t_B is approximately 6-fold lower then the highest endogenous levels of C_B.

It follows from (b) and (c) that the response of B to endogenous stimulation is not full. However, high exogenous bolus of B elicits dose-dependant release of B-secretion at levels higher then the typical endogenous B-concentration. It is easy to see that due to (b) the maximal endogenous B concentration is less than 125. Due to the choice of t_B (see (d)), B almost fully suppresses the release of A between pulses, which in turn results in low intervolley B-secretion.

To simulate the network from Fig. 2, right panel (A is an inhibitor) we use the following reference system of delayed ODEs

$$\begin{aligned} \frac{dC_A}{dt} &= -1C_A(t) + 50\frac{[C_B(t-3)/20]^2}{[C_B(t-3)/20]^2+1} \\ \frac{dC_B}{dt} &= -2C_B(t) + 500\frac{1}{[C_A(t)/5]^2+1} \end{aligned} \qquad (18)$$

The system parameter a in Eq. (17) was increased 10-fold (compared to Eq. (18)) to guarantee existence of a periodic solution.

SIMULATION OF FEEDBACK EXPERIMENTS. The success of a modeling effort is frequently measured by the capability of the construct to reproduce pivotal feedback experiments. Accordingly, we discuss the correct way of modeling and

the system reaction to three common experimental techniques, aimed to disclose the specific linkages within an endocrine system.

Antibody infusion

The introduction of an antibody (Ab) to a certain substance, referred here as S, is generally accompanied by a transformation of S, which results in effectively removing S from the system. The removal rate depends on the specific chemical reaction between Ab and S, and increasing the elimination rate of S (corresponding to the pool where Ab is administered) is a modeling approach that works in most cases. However, note that the reaction specifics may result in change of the single half-life pattern into a multiple half-life model, but the single half-life approximation still might be sufficient in the simulations.

To exemplify the idea we simulated variable removal of the inhibitor A in the reference model described by Eq. (18). Three simulations were performed, in which the coefficient β was increased 2-fold (left), 6-fold (middle), or 15-fold (right) at time $t = 75$.

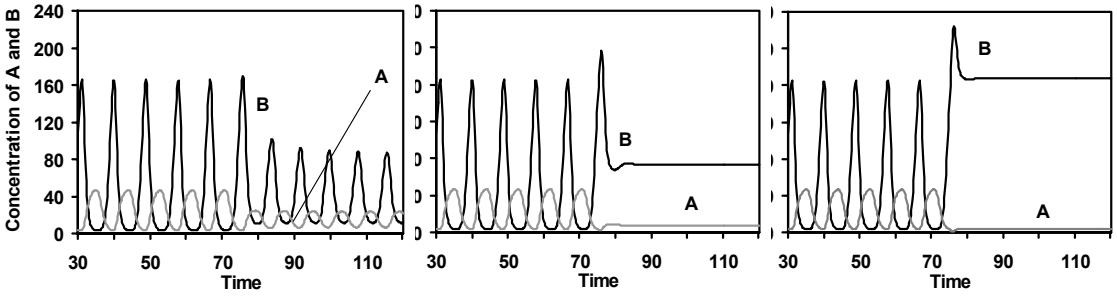

Fig. 5. Simulated variable infusion (starting at t=75) of antibody to the inhibitor A in the reference model outlined in Eq. (18). The plots depict low (left panel), medium (middle panel), or almost complete (right panel) removal of A.

The plots in Fig. 5 (left panel) capture a very interesting phenomenon predicted by the model: the decrease in the peak amplitudes of B, even though an inhibitor is removed from the system. In the current model, this is explained by the actual increase of the rate at which A initiates its rise and reaches its action threshold, which, in turn, promotes an earlier suppression of B-secretion.

Sensitivity modification

Modifying the profiles of the control function models alterations in system sensitivity. For example, if the sensitivity of certain cell group depends on the number of opened receptors, we could simulate receptor blockage/stimulation via changing the parameters of the corresponding control function. In the model described in Eq. (17), this would correspond to changes in the threshold, or in the Hill coefficient. Reducing (increasing) a threshold results in sensitivity increase (decrease). Changes in the Hill coefficient affect the slope of the control function. In general, increasing the Hill coefficient slightly changes the frequency and the amplitude, without affecting the pulsatility character of the profiles. In contrast, a

decrease could effectively disturb the oscillations by preventing the system to overshoot the steady state.

We illustrate the effect of changing all thresholds and Hill coefficients in Eq. (17): Fig. 6.

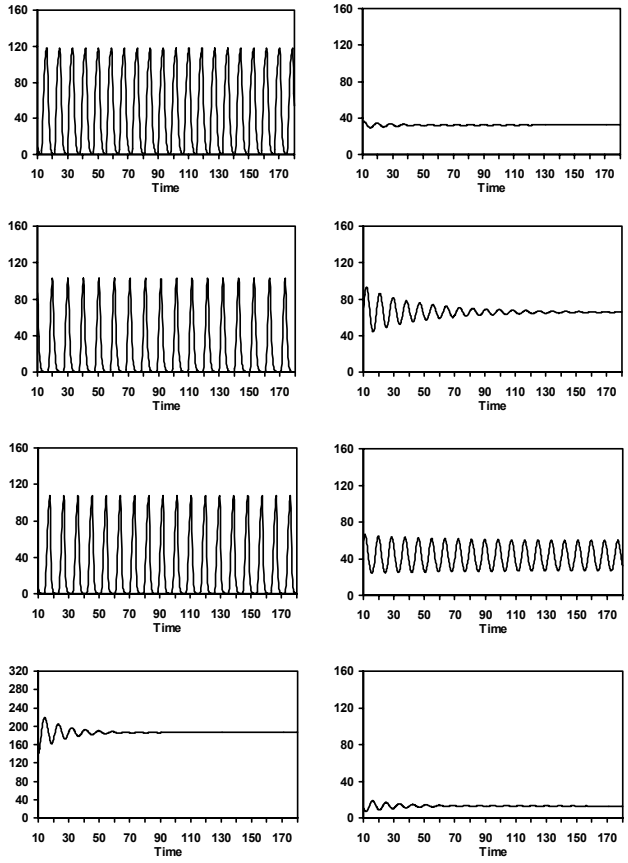

Fig. 6. Model response to alterations in system sensitivity. All profiles depict the dynamics of $C_B(t)$. A: Changing n_B from 2 to 10 (left) and to 1 (right); B: Changing t_B from 20 to 0.2 (left) and to 80 (right); C: Changing n_A from 2 to 20 (left) and to 2/3 (right); D: Changing t_A from 5 to 1/40 (left) and to 15 (right).

An increase in n_B or n_A (Fig. 6, A, C, left panels) produced a slight change in the frequency and amplitude. Decrease in n_B or n_A resulted in pulse shrinking (Fig. 6, C, right panel) or in lost of periodicity (Fig. 6, A, right panel) if the control functions can no longer provide the necessary inertia for overshooting the steady state value. Increasing T_B from 20 to 80 (Fig. 6, B, right panel) results in a condition in which B cannot exert the necessary suppression on A. The concentration of B is limited from above and increasing its action threshold gradually obliterates the effect of the delay containing term. Decreasing T_B to 0.2 has no visual effect on the simulated profiles (Fig. 6, B, left panel). The pulsatility is not affected because the suppressive action of B on A is not modified. It only starts somewhat earlier, but there is still a 3-hour delay in this action, which, in this particular model, is sufficient to maintain oscillations. The

analysis of the effect produced by changes in T_A is somewhat different. Both, increasing and decreasing might affect the oscillations. When T_A is decreased, even small amount of A is sufficient to produce a full response, which obliterates the pulsatility (Fig. 6, D, left panel). The fact that the concentration of A is bounded from below independently of T_A is crucial (Eq. (14)). Increasing T_A results in left shift of the control function S_B, thus, preventing A from stimulating B, which in turn reduces the oscillations (Fig. 6, D, right panel).

A more formal approach to explaining the reduction in the range of the oscillations (the "shrinking" of the profile) would consist of (recursive) application of the inequalities Eq. (14). For example, from the right hand side of Eq. (14) it is evident that if $T_A \to 0$ then $C_B \to b/\beta$ and if $T_B \to \infty$ then $C_A \to a/\alpha$.

Exogenous infusion.

The correct way to simulate exogenous infusion of a hormone, which is also a system node, would be to add an infusion term to the right-hand side of the corresponding ODE. This term should correspond to the infusion rate profile in the real experiment. Mathematically, it might be interpreted as change in the basal secretion. In terms of the specific model described by Eq. (11), if we are simulating infusion of hormone B, the corresponding equation changes as follows:

$$\frac{dC_B}{dt} = -\beta C_B(t) + S_B(C_A(t - D_A)) + \inf(t), \tag{19}$$

where $\inf(t)$ is the infusion rate term. The solution of the above equation is the sum of both endogenous and exogenous concentrations of B. To follow the distinction explicitly, a new equation should be added to the system:

$$\frac{dC_{\inf}}{dt} = -\beta C_{\inf}(t) + \inf(t)$$

and $C_B(t)$ has to replaced by $C_B(t) + C_{\inf}(t)$ in all model equations, except the one that describes the rate of change of the concentration of B. To sum up, the core equations are

$$\begin{aligned} \frac{dC_A}{dt} &= -\alpha C_A(t) + S_A\{[C_B + C_{\inf}](t - D_B)\} \\ \frac{dC_B}{dt} &= -\beta C_B(t) + S_B(C_A(t - D_A)) \\ \frac{dC_{\inf}}{dt} &= -\beta C_{\inf}(t) + \inf(t) \end{aligned} \tag{20}$$

The model above (Eq. (20)) is in essence a 3-node/1-feedback construct, where exogenous B is the new node. A particular example, illustrating infusion simulation is shown later in this section (see "IDENTIFYING NODES, CONTROLLING THE OSCILLATIONS").

OSCILLATIONS GENERATED BY A PERTURBATION. In the reference models from the previous section the pulsatility was generated by a system that has a unique

periodic solution and a unique fixed repelling point. The purpose of this section is to demonstrate, that oscillations may occur as a result of disrupting a system that does not have a periodic solution, and its fixed point is an asymptotically stable focus (Fig. 3, A).

We illustrate this concept on an earlier example. Fig. 6 (B, right panel) depicts the profile of the solution to the following delayed ODE

$$\begin{aligned} \frac{dC_A}{dt} &= -1C_A(t) + 5\frac{1}{[C_B(t-3)/80]^2 + 1} \\ \frac{dC_B}{dt} &= -2C_B(t) + 500\frac{[C_A(t)/5]^2}{[C_A(t)/5]^2 + 1} \end{aligned} \qquad (21)$$

The difference between this model and the reference construct (Eq. (17)) is in the 4-fold increase of the threshold T_B. In this case, there is no periodic solution and the unique fixed point attracts all trajectories in the phase space. Therefore, this system by itself cannot generate stable oscillations. However, if it is externally stimulated it can be removed from its steady state and oscillations will be detected. For example, assume that at $t = 350$ the secretion of B was briefly suppressed. This removes the trajectory in the phase space away from the fixed point and the system would have enough energy to initiate another waning pulse sequence: Fig. 7, left panel. Moreover, if we allow for some periodic external control on the secretion, the hormone profile displays sustained pulsatility with bursts of variable amplitude: Fig. 7, middle panel. The frequency of the pulses is controlled by the coefficients of the core system Eq. (21), while the peak amplitudes follows the external stimulus.

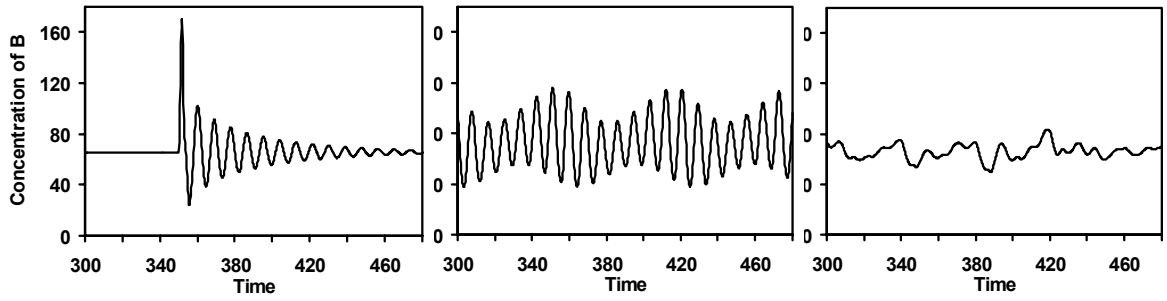

Fig. 7. Oscillations generated by perturbations of the system in Eq. (21). The left plot depicts a brief suppression of the secretion of B at t=350. The rest profiles depict external periodic (middle panel) or random (right panel) control on the coefficient b, which determines the release of B.

If the perturbation is random, it generates pulses of approximately the same frequency as in the previous cases, but with highly variable amplitudes. In the simulation presented in Fig. 7 (right panel) we superimposed 40 % Gaussian noise on the parameter b. Even though some peaks cannot be detected an overall periodicity (the same as in Fig. 7, left and middle panels) is apparent.

In the above examples, the perturbation was assumed to be external and independent of the core system. Later on, we show that a delayed system feedback could also provide enough energy and trigger oscillations in sub-models with damped periodicity. In the three-node example from *"Networks with*

multiple feedback loops" a 2-node sub-system (with no direct delay in its feedback, and therefore, without a periodic solution) is perturbed by a delayed system loop via the third node. This removes the whole system from its steady state and drives consecutive pulses during recurrent volleys.

IDENTIFYING NODES, CONTROLLING THE OSCILLATIONS. When the hormone A cannot be measured directly and is an inhibitor (the network in Fig. 2, right panel) we can test whether it is involved in generating the oscillations of B by neutralizing the action (A-receptor blocker) or by removing (antibody infusion) A from its action pool. On the other hand, if A is a stimulator (Fig. 2, left panel) a large constant infusion of A should remove the oscillations (by exceeding the action threshold, resulting in continuous full response from the target organ). This concept is exemplified in Fig. 8, which depicts two computer-generated predictions for the system response to exogenous infusion of hormone A (assuming that A stimulates B, Eq. (18)). We simulated constant low (left panel) and high (right panel) infusion of A by increasing the basal A-secretion from zero to two different levels, starting at t=75.

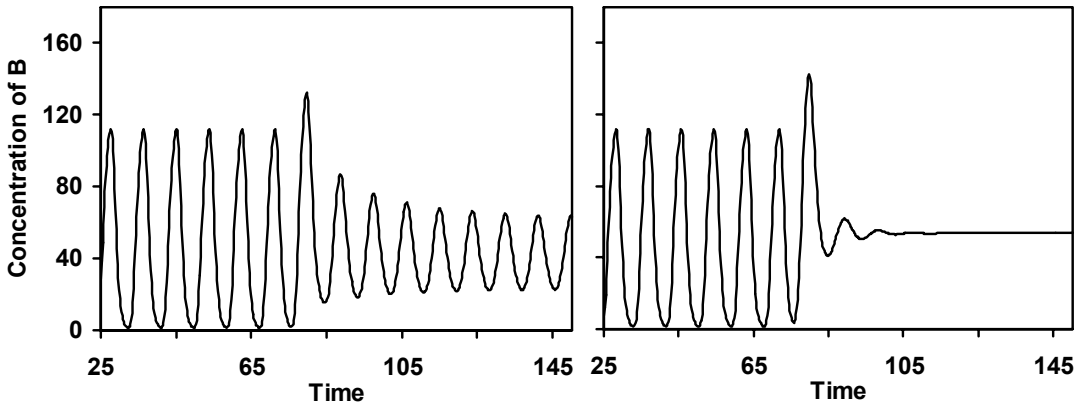

Fig. 8. System response (Eq. (22)) to exogenous infusion of A. The plots show simulation of constant low (left panel) and high (right panel) infusion of A starting at t=75.

The model predicts gradual pulse "shrinking" towards the current steady state level. If the exogenous administration of A is sufficiently high (right panel) the pulses wane and the secretion becomes constant. The profiles in Fig. 8 depict the numerical solution (concentration of hormone B) of the system

$$\begin{aligned} \frac{dC_A}{dt} &= -C_A(t) + \mathrm{Inf}(t) + 5\frac{1}{[C_B(t-3)/20]^2 + 1} \\ \frac{dC_B}{dt} &= -2C_B(t) + 500\frac{[C_A(t)/5]^2}{[C_A(t)/5]^2 + 1} \end{aligned} \qquad (22)$$

with two different continuous infusion terms satisfying:

$$\mathrm{Inf}(t) = \begin{cases} 0 & \text{if } t \le 75 \\ 1 \text{ or } 2 & \text{if } t \ge 76 \end{cases}$$

The parameters and control functions were chosen arbitrarily to simulate a network like the one in Fig. 2 (left panel), which generates stable oscillations.

Almost identical results (see Fig. 5) can be achieved by simulating partial or complete removal of A in the case when A is an inhibitor (the network from Fig. 2, right panel). This should be done by increasing the rate of elimination of A to simulate additional removal due to infusion of antibody (see "SIMULATION OF FEEDBACK EXPERIMENTS" for details).

However, these experiments cannot disclose whether A is actually involved in a feedback with B, or acts merely as a trigger to remove a certain sub-system from its steady state. For example, consider the two networks shown in Fig. 9 and suppose that only the concentrations of hormone B can be measured.

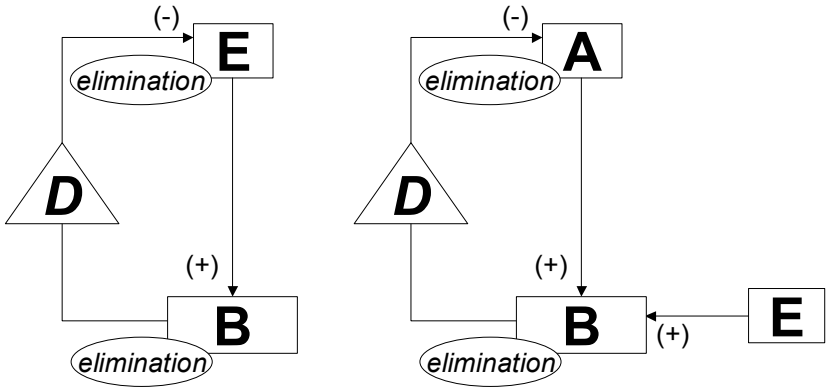

Fig. 9. Two hypothetical networks, in which a hormone E stimulates the secretion of B. E is either involved in a delayed feedback (left panel), or removes the sub-system A-B (right panel) from its steady state.

Assume that E stimulates B, and its removal obliterates the secretion of B. Since E cannot be measured, we have no direct means to establish whether E is involved in a delayed feedback loop with B. Moreover, in both networks, constant high infusion of E (as proposed above) removes the pulsatility and elicits constant secretion of B. Therefore, a more sophisticated experiment is required to reveal whether E is indeed involved in a feedback loop with B (Fig. 9, left panel) or acts by perturbing the A-B sub-system (Fig. 9, right panel). A possible approach would include blocking the exogenous E secretion with subsequent introduction of a single endogenous E bolus. The system response would be a single spike of B secretion, if the network were that, depicted on Fig. 9 (left panel), or a waning train of several B pulses if the network is the one, shown on Fig. 9 (right panel). Most importantly, the required suppression of endogenous E release might be achieved by a constant high infusion of B (or B-analog), which should be distinguishable from endogenous B-secretion.

SEPARATING SYNTHESIS FROM SECRETION. In certain cases, it would be appropriate to separate on a network level the hormone synthesis from its release. This would be important if certain compound differently affects these processes. For example, let consider again the network from Fig. 2, left panel, in an attempt to

explain a rebound release of B following a withdrawal of continuous infusion of certain substance C. Assume that during the infusion of C the release of B was suppressed and that we have evidence that C is not affecting the release of A. Possible explanation of the rebound phenomenon would be that C affects the release of B, but not its synthesis. However, since all conduits in the network are affected in this experiment, the intuitive reconstruction of all processes involved is not trivial. The simulation requires introduction of a "storage" pool in which B is synthesized and packed for release and another pool (e.g. circulation) in which B is secreted. This adds a new equation to the model, describing the dynamics of the concentration of B in the storage pool. The following assumptions would be appropriate:

1. The concentration of B in the storage pool (P_B) is positively affected by the synthesis and negatively affected by the release.
2. The concentration P_B exerts a negative feedback on the synthesis of B and cannot exceed a certain limit $P_{\max}$.
3. The rate of release of B from the storage pool is stimulated by the storage pool concentration but might be inhibited by the concentration of B in the exterior.
4. B is subjected to elimination only after it is secreted

In order to provide an abstract example, let assume that in the network from Fig. 2 (left) we have in addition to A and B a new substance C, that inhibits the secretion (competing with A), but does not affect the synthesis of B: Fig. 10.

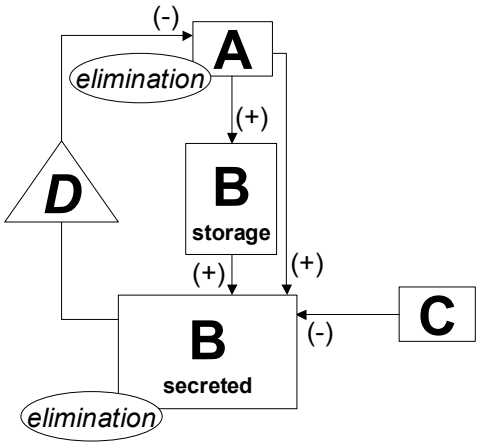

Fig. 10. Formal network depicting the system distinction between synthesis and release. C suppresses the release of B, but not its synthesis.

Using Eq. (10) as a suitable form for the "competitive" control function, we can describe the network by the following system of delayed ODEs:

$$
\begin{aligned}
\frac{dC_A}{dt} &= -\alpha C_A(t) + a\frac{1}{(C_B(t-D_B)/T_B)^{n_B}+1} \\
\frac{dC_B}{dt} &= -\beta C_B(t) + b\frac{(C_A(t)/T_{A,1})^{n_{A,1}}}{(C_A(t)/T_{A,1})^{n_{A,1}}+(C_C(t)/T_C)^{n_C}+1}\frac{(P_B(t)/T_P)^{n_P}}{(P_B(t)/T_P)^{n_P}+1} \\
\frac{dP_B}{dt} &= c(P_{\max}-P_B)\frac{(C_A(t)/T_{A,2})^{n_{A,2}}}{(C_A(t)/T_{A,2})^{n_{A,2}}+1} - b\theta\frac{(C_A(t)/T_{A,1})^{n_{A,1}}}{(C_A(t)/T_{A,1})^{n_{A,1}}+(C_C(t)/T_C)^{n_C}+1}\frac{(P_B(t)/T_P)^{n_P}}{(P_B(t)/T_P)^{n_P}+1}
\end{aligned}
\tag{23}
$$

Here, for simplicity, we assumed that circulating B levels do not feedback on the secretion. This would correspond to a model with much higher concentration in the storage pool than in the circulation. In the above presentation c controls the rate of A-stimulated synthesis of B. The parameter θ represents the ratio between the volumes of the storage pool and the pool in which B is secreted. Typically, the second pool is larger and $\theta > 1$. We have supposed that the control functions, which correspond to the A-driven synthesis and release are different with distinct thresholds $T_{A,1}$ and $T_{A,2}$, and corresponding Hill coefficients $n_{A,1}$ and $n_{A,2}$. The control, exerted on the secretion by the current concentrations of B in the storage pool, is presented by the up-regulatory function $\frac{(P_B(t)/T_P)^{n_P}}{(P_B(t)/T_P)^{n_P}+1}$. The following values were assigned to the parameters that appear in Eq. (23):

$$
\begin{aligned}
&\alpha = 1; \quad \beta = 2; \quad \theta = 6; \quad a = 4; \quad b = 4000; \quad c = 2; \quad P_{\max} = 1000; \\
&T_{A,1} = 4; \quad T_{A,2} = 3; \quad T_B = 40; \quad T_C = 10; \quad T_P = 500; \\
&n_{A,1} = 2; \quad n_{A,2} = 2; \quad n_B = 2; \quad n_C = 2; \quad n_P = 2;
\end{aligned}
$$

The infusion term $C_C(t)$ is assumed to be a non-zero constant only during the time of infusion:

$$
C_C(t) = \begin{cases} 0 & \text{if} \quad t < 55 \\ 500 & \text{if} \quad 56 < t < 95 \\ 0 & \text{if} \quad t > 96 \end{cases}
$$

The model output is shown in Fig. 11 and the plots clearly demonstrate a B-rebound following the withdrawal of C (Fig. 11, left panel).

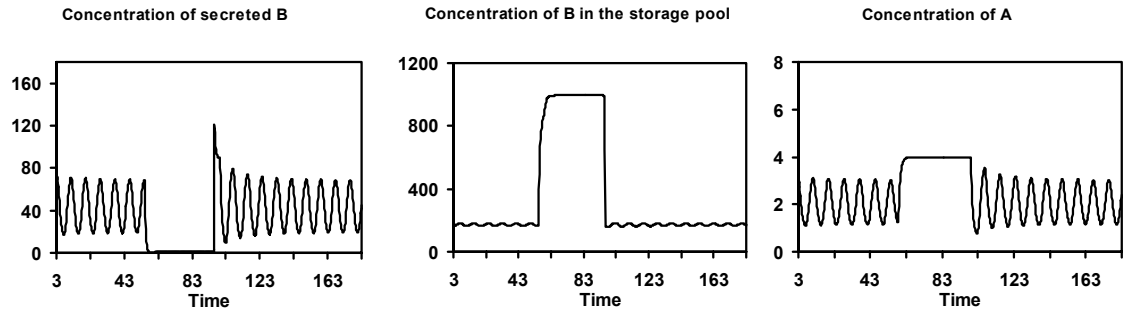

Fig. 11. Simulated rebound response following a withdrawal of continuous C-infusion (timeline 55 - 95). Left panel: concentration of secreted B (in the circulation). Middle panel: concentration of B in the storage pool. Right panel: A-concentration dynamics.

During the infusion the secretion of B is blocked, but not the synthesis and the concentration in the storage pool is elevated (Fig. 11, middle panel). The concentration of A increases (Fig. 11, right panel), since low B levels cannot effectively block its release. Thus, the model explains the rebound jointly by the augmented concentration in the storage pool and the increased secretion of A.

Networks with multiple feedback loops

The available experimental data might suggest that the release of a particular hormone B is controlled by multiple mechanisms, with different periodicity in the timing of their action. This implies that probably more than one (delayed) feedback loops regulate the secretion of B and the formal endocrine network may include more than two nodes. In determining the elements to be included in the core construct, it is important to keep track on the length of the delays in the feedback action of all nodes of interest. For example, if the goal were to explain events recurring every 1 to 3 hours, the natural candidates to be included in the formal network would be nodes, involved in feedback or feed-forward relations with B with delays shorter than 3 hours. Long feedback delays cannot account for high frequency events. In particular, if we hypothesize that a certain delayed feedback is responsible for a train of pulses in the hormone concentration profile, the direct delay must be shorter than the interpulse interval.

In this section we briefly discuss some features of abstract endocrine networks, incorporating more than one delayed feedback loops. Each loop accounts for its own oscillator mechanism and in what follows, we consider networks with two (delayed) feedback loops. Examples of 2-feedback constructs are shown in Fig. 12

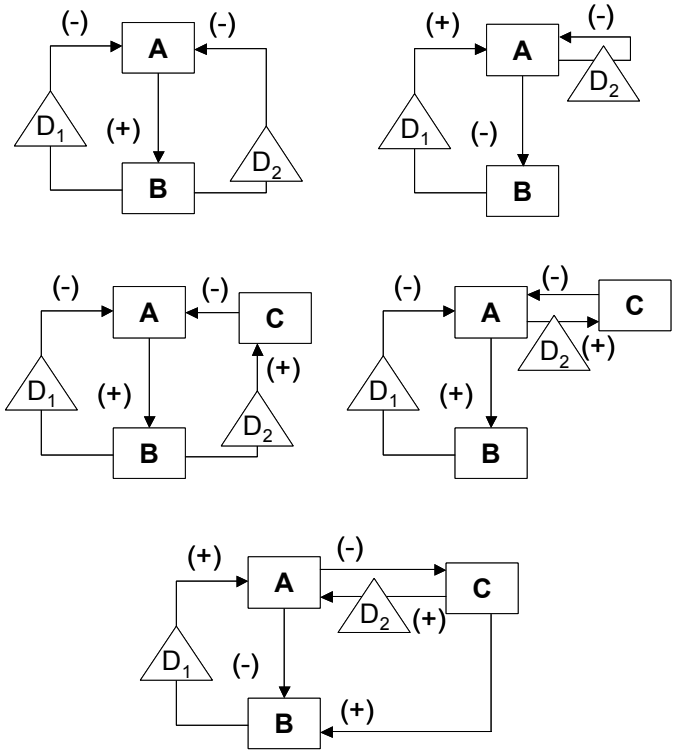

Fig. 12. Examples of hypothetical endocrine networks with more than one delayed feedback loops.

It should be noted, that each of the two 3-node networks, shown in the middle panels of Fig. 12, could be reduced to its corresponding 2-node network from the top panels of Fig. 12. For example, let consider the 3-node/2-feedback network shown in Fig. 12, middle left panel. Assuming that both B and C can fully suppress the release of A, we can describe the formal network by the system of delayed ODE:

$$
\begin{aligned}
\frac{dC_A}{dt} &= -3C_A(t) + 10000\frac{1}{[C_B(t)/100]^3 + 1}\frac{1}{[C_C(t)/70]^{20} + 1} \\
\frac{dC_B}{dt} &= -2C_B(t) + 6000\frac{[C_A(t)/500]^{40}}{[C_A(t)/500]^{40} + 1} \qquad (24) \\
\frac{dC_C}{dt} &= -3C_C(t) + 180 + 1320\frac{[C_B(t-1.5)/200]}{[C_B(t-1.5)/200] + 1}
\end{aligned}
$$

Here, for simplicity, we have assumed that there is no delay in the feedback B $\rightarrow$ A. This system is capable of generating recurring multiphase volleys, by the mechanism described in "OSCILLATIONS GENERATED BY A PERTURBATION": Fig. 13.

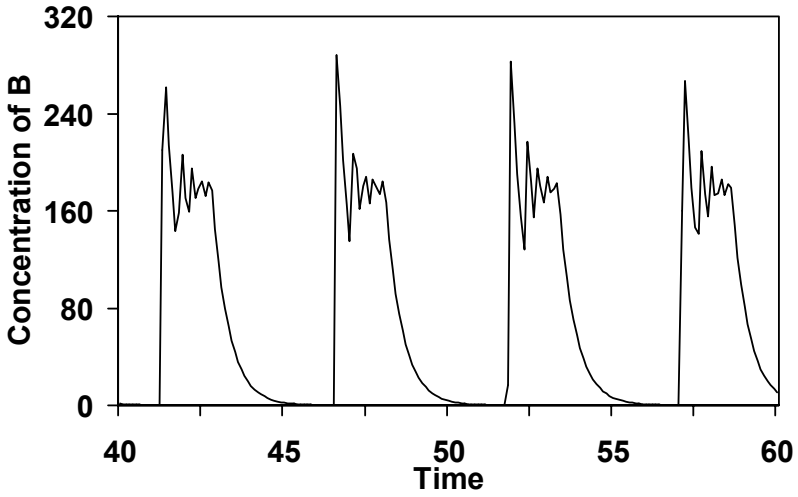

Fig. 13. Computer-generated output (concentration of B) of the core system Eq. (24).

However, analogous results can be achieved by reducing the 3-node network to a 2-node model with two feedbacks. In fact, the sequence of nodes and conduits B $\rightarrow$ C $\rightarrow$ A $\rightarrow$ B is, in essence, a negative 2-node delayed feedback loop: B $\rightarrow$ A $\rightarrow$ B. Therefore, it can be modeled in the usual way (by simply removing C from the system). The reduced network is the one shown in Fig. 12, upper left panel.

A corresponding simplified system of delayed ODEs could be

$$\frac{dC_A}{dt} = -3C_A(t) + 10000\frac{1}{[C_B(t)/100]^3 + 1}\frac{1}{[C_B(t-1.5)/50]^3 + 1}$$

$$\frac{dC_B}{dt} = -2C_B(t) + 6000\frac{[C_A(t)/500]^{40}}{[C_A(t)/500]^{40} + 1}$$

and the model output (not shown), even without any special efforts to adjust the system parameters, is almost identical to the profile shown in Fig. 13.

Decreasing the number of equations from three to two reduces the number of parameters to be determined and the time needed for solving the equations numerically. This would be most important if multiple computer runs are required. Therefore, adding the third node in the formal network can be justified only if the goal is to simulate experiments, involving C explicitly. And even then, the initial adjustment of the model would be significantly facilitated if C enters the system after the 2-node construct is validated.

Note, that if the network is more complex, the attempt to reduce the number of nodes might not be beneficial. For example, the network shown in Fig. 12, lower panel, cannot be transformed into a 2-node model, due to the high system interconnectivity. We comment more on this in the next section.

Summary and discussion

The mathematical methods presented in this chapter are tailored to quantitatively interpret formal endocrine networks with (delayed) feedbacks. The main goal is to illustrate different conditions, under which oscillations can emerge.

The formal network itself, consists of nodes and conduits, and is based on a qualitative analysis of available experimental data[7]. In our presentation the nodes are hormone concentrations in abstract pools, in which hormones are released or synthesized, under the control of other hormones. The conduits specify how the nodes interact within the network. The quantitative analysis of the formal network is based on approximation of the rate of change of a single system node. This essentially means that the dynamics of the hormone concentration is described with a single (delayed) ordinary differential equation (ODE). To this end, we assume that the rate of change of hormone concentration depends on two processes - secretion and ongoing elimination. We work with single half-life elimination model and express the control of the synthesis as a combination of sigmoid Hill functions, depending on the related nodes. The derivation of the ODE is demonstrated, along with a brief analysis of the properties of its solution to facilitate the actual determination of all system parameters.

The formal network is then interpreted as a dynamic system by combining all ODEs that describe system nodes dynamics. We exemplify the ideas on a 2-node/1-feedback model - one of the simplest meaningful examples of a network capable of generating and sustaining periodic behavior. In fact, a variety of systems display oscillatory behavior, driven by a single feedback loop. The simplest case is a 1-node/1-feedback network, in which a hormone after being secreted suppresses its own release, immediately or after some lag time. This system can generate periodic behavior, only if the delay in the feedback is greater than zero. We do not discuss this case here.

A network may incorporate a single feedback loop in a more complex way, e.g. via a combination of two or more nodes. For example, simple stability analysis of the steady state shows that a 3-node/1-feedback network is capable of sustaining periodicity even without a delay in the feedback loop and relatively low Hill coefficients[8,13]. However, for a variety of practical cases, it is feasible to reduce the 3-node/1-feedback network to a 2-node/1-feedback construct as shown in the previous section.

Some specifics in endocrine network modeling are exemplified on two 2-node/1-feedback networks, in which the concentration of one hormone regulates the secretion of another, which in turn controls the release of the first hormone. This construct could generate oscillations even if there is no explicit delay in the feedback. However, it will be a damped periodicity, since the oscillations will fade and approach the steady state of the system. In contrast, a non-zero delay combined with a sufficiently large nonlinearity in the control functions (high Hill coefficients) guarantees steady periodic behavior, as all trajectories approach a non-trivial limit cycle.

We relate all parameters to their physiological meaning and analyze the solutions to our reference systems, which always have only one fixed point (steady state), which is either a repellor, or an attractor (Fig. 3). In the first case the system has a unique limit cycle – a periodic solution, which attracts all trajectories in the phase space and, thereby generates stable periodic behavior

[13] J. Richelle, *Bull. Cl. Sci. Acad. R. Belg*. **63**, 534 (1977)

(Fig. 4). In the second case, the steady state is either a focus or a node and attracts all trajectories in the phase space. Therefore, the construct displays damped periodic behavior. In particular, if it is in a state close to the fixed point an external perturbation initiates a waning train of pulses (Fig. 7, upper left panel). Therefore, oscillations might be generated even by a system that does not have a periodic solution, and its fixed point is asymptotically stable. However, an external source of perturbations must be provided. Note, that the frequency of the oscillations is largely independent of the external perturbation (Fig. 7).

We use the two reference systems to illustrate the modeling of three common experimental techniques: infusion of antibody to one of the nodes, sensitivity alterations, and exogenous infusion of one of the system hormones. We comment on the correct way to perform these approximations and examine the corresponding model response. In particular, the simulations illustrate conditions that might disrupt the periodicity.

Increasing the elimination rate of a hormone simulates infusion of antibody, and almost a complete removal of one of the nodes, results in loss of periodicity (Fig. 5). Changes in the profiles of the control functions model alterations in system sensitivity. The analysis shows that if a model has a stable periodic behavior, the increase in one of the Hill coefficients would not change the system performance: Fig. 6, A & C, left panels (see also[14]). On the other side, a decrease in the same parameter may transform the steady state from a repellor into an attractor and affect the periodic behavior. Changes in the action thresholds may also affect the periodicity: Fig. 6, B & D. Exogenous infusion can be simulated by a simple increase in the basal secretion, or by introducing a third node, in case we would like to distinguish between exogenous infusion and endogenous secretion of one and the same substance (Eqs. (19), (20)).

We illustrate how these experiments may be used to disclose, whether certain hormone A is involved in generating the oscillations of another hormone B. The idea is to alter A in such way that the periodic B-profile is transformed into a constant non-zero secretion. When A inhibits B, we can neutralize its action (receptor blocker) or remove (antibody) it from the system. In the later case the model predicts that the periodicity disappears and is replaced by a stable B-secretion (Fig. 8). Alternatively, if A stimulates B, a large continuous A-infusion obliterates the oscillations by exceeding the action threshold, and eliciting a unvarying full B-response from the target organ (Fig. 5). Additionally, the model provides means to disclose whether A is actually involved in a feedback loop with B or generates oscillations by perturbing another subsystem (see "IDENTIFYING NODES, CONTROLLING THE OSCILLATIONS").

To be able to capture a variety of feedback systems we separate on a network level the hormone synthesis from its release. The proper simulation requires a new "storage" pool in which the hormone is synthesized and stored, in addition to the pool, in which the hormone is secreted. We used this distinction to provide plausible explanation of a rebound release, following withdrawal of an agent that suppresses the secretion, but not the synthesis.

[14] L. Glass and S.A. Kauffman, *J. Theor. Biol.*, **39**, 103 (1973)

We would like to emphasize the importance of keeping the model as minimal as possible while performing the initial qualitative analysis of the available experimental data. In general, formal endocrine networks might incorporate multiple feedbacks loops and nodes. However, long feedback delays cannot account for high frequency events. Therefore, if the model attempts to explain pulses of a hormone that recur every H hours, it might be sufficient to include in the formal network only feedback loops with delay shorter than H. Moreover, if a feedback loop enters the network via a multiple-node subsystem, it might be possible to reduce the number of nodes and simplify the model without affecting its performance. The example, provided in the previous section demonstrates a case, in which we could safely remove a "passive" node from a feedback loop, and still retain the overall periodic behavior.

Unfortunately, we cannot always reduce complex networks. The model shown in Fig. 12, lower panel, is an example in which the system interconnectivity would not allow any simplification. Complex networks with intertwined feedback loops are considered in [2,3] and their analysis strongly depends on the specific physiology. It should be noted that in this chapter we do not consider more complicated cases, like for example, networks that have multiple steady states of different type, which is a significant complication. Such systems can be approached in the early stage of their analysis by Boolean formalization[15,16] which serves as an intermediate between modeling phases (b) and (c) described in the first section. This method describes complex systems in simple terms and allows for preliminary finding of all stable and unstable steady states.

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