Foliation-Based Parameter Tuning in a Model of the GnRH Pulse and Surge Generator

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Abstract. We investigate a model of the GnRH pulse and surge generator, with the definite aim of constraining the model GnRH output with respect to a physiologically relevant list of specifications. The alternating pulse and surge pattern of secretion results from the interaction between a GnRH secreting system and a regulating system exhibiting slow-fast dynamics. The mechanisms underlying the behavior of the model are reviewed from the study of the Boundary-Layer System according to the “dissection method” principle. Using singular perturbation theory, we describe the sequence of bifurcations undergone by the regulating (FitzHugh–Nagumo) system, encompassing the rarely investigated case of homoclinic connection. Based on pure dynamical considerations, we restrict the space of parameter search for the regulating system and describe a foliation of this restricted space, whose leaves define constant duration ratios between the surge and the pulsatility phase in the whole system. We propose an algorithm to fix the parameter values also to meet the other prescribed ratios dealing with amplitude and frequency features of the secretion signal. We finally apply these results to illustrate the dynamics of GnRH secretion in the ovine species and the rhesus monkey.

Key words. coupled oscillators, bifurcations, slow-fast dynamics, amplitude and frequency control, ovulation, neuroendocrinology, GnRH pulsatility, GnRH surge

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1. Introduction. In vertebrates, the reproductive system is made up of the hypothalamus, belonging to the central nervous system, the pituitary gland, and the gonads (ovaries in females and testes in males). Within the hypothalamus, specific neurons secrete the GnRH (Gonadotropin Releasing Hormone) in a pulsatile manner. This pulsatility has a fundamental role in the differential control of the secretion of both gonadotropins by the pituitary gland: LH (luteinizing hormone) and FSH (follicle stimulating hormone). In females, the pulsatile pattern is tremendously altered once per ovarian cycle into a surge which triggers LH surge and ovulation in response to increasing levels of estradiol secreted by the ovaries. The estradiol signal is conveyed to GnRH neurons through a network of interneurons. The balance between stimulatory and inhibitory signals emanating from interneurons controls the behavior of the GnRH network.

We have proposed a mathematical model accounting for the alternating pulse and surge pattern of GnRH secretion [5]. The model is based on the coupling between two systems
running on different time scales. We thus consider the following four-dimensional dynamical system:

\[
\begin{align*}
\epsilon \delta \frac{dx}{dt} &= -y + f(x), \\
\epsilon \delta \frac{dy}{dt} &= a_0 x + a_1 y + a_2 + cX, \\
\epsilon \gamma \frac{dX}{dt} &= -Y + g(X), \\
\frac{dY}{dt} &= b_0 X + b_1 Y + b_2,
\end{align*}
\]

where \( f \) and \( g \) are two cubic functions:

\[
\begin{align*}
f(x) &= \nu_0 x^3 + \nu_1 x^2 + \nu_2 x, \\
g(X) &= \mu_0 X^3 + \mu_1 X^2 + \mu_2 X.
\end{align*}
\]

Following [5], we assume throughout the paper that all parameter values are positive except \( \nu_0 \) and \( \mu_0 \).

The faster system (1.1a)–(1.1b), henceforth named the “GnRH Secreting System,” corresponds to the average activity of GnRH neurons, while the slower one (1.1c)–(1.1d), named the “Regulating System,” corresponds to the average activity of regulatory neurons. The \( x, X \) variables represent the neuron electrical activities (action potential), while the \( y, Y \) variables relate to ionic and secretory dynamics. In each system, the fast and slow variables feedback on each other. The coupling between both systems is mediated through the unilateral influence of the slow regulatory interneurons onto the fast GnRH ones (\( cX \) term in (1.1b)). The coupling term aggregates the global balance between inhibitory and stimulatory neuronal inputs onto the GnRH neurons. The analysis of the slow-fast dynamics exhibited within and between both systems allows us to explain the different patterns (slow oscillations, fast oscillations, and periodical surge) of GnRH secretion.

This model can be used as a basis for understanding the control exerted by ovarian steroids (estradiol and progesterone) on GnRH secretion, in terms of amplitude and frequency of oscillations, and for differentiating a direct action (on the GnRH network) from an indirect action (on the regulatory network) of steroid feedbacks. To account accurately for this control, we have to fully understand the sequence of bifurcations corresponding to the different phases of GnRH secretion and the occurrence of a hysteresis loop. Our main goal in this paper is thus to carry on a deeper analysis of this model, based on singular perturbation theory, to meet precise quantitative neuroendocrinological specifications. These specifications deal with the duration of the luteal (progesterone-dominated) and follicular (estradiol-dominated) phases of the ovarian cycle, and the ratios between (i) the surge duration and the whole cycle duration, (ii) the pulse amplitude and the surge amplitude, and (iii) the increase in pulse frequency from the luteal to the follicular phase.

The paper is organized as follows. In section 2, we recall the main properties of the original model and explain the mechanisms underlying the alternation between pulsatility phases and surges in the secretion signal. In section 3, we give a precise bifurcation diagram of the
Regulating System as a function of the parameters $\varepsilon$, $b_1$, and $b_2$, which allows us to restrict the domain of parameter values. We prove the existence and describe the shape of a foliation of the restricted domain, whose leaves correspond to constant duration ratios. This novel type of control form enables us to find the parameter values which simultaneously yield any prescribed value for each of the different ratios considered. Section 5 deals with the influence of other parameters on the remaining specifications. It calls on methodological developments falling into the field of weakly coupled nonlinear oscillators. Finally, in section 6, we enunciate in detail the quantitative specifications for the ovarian cycle in the sheep and rhesus monkey, since direct measurements of GnRH are available in these species. In each case, we describe the search for the relevant parameter values and illustrate the resulting GnRH secretion signal from numerical simulations.

2. Qualitative understanding of the original model. In this section, we recall the main mechanisms underlying the model behavior, which is necessary to introduce the subsequent analysis in section 3.

2.1. Reparameterization. Some parameters of (1.1) are useless since, whatever their values, we can fix the other parameter values to obtain the same orbit. With the simple rescaling

$$\tilde{t} = b_0 t,$$

the system (1.1) reads

\begin{align*}
\varepsilon' \delta' \frac{dx}{dt} &= -y + f(x), \\
\varepsilon' \frac{dy}{dt} &= a_0' x + a_1' y + a_2' + c' X, \\
\varepsilon' \frac{dX}{dt} &= -Y + g(X), \\
\varepsilon' \frac{dY}{dt} &= X + b_1' Y + b_2',
\end{align*}

where

$$
\begin{align*}
a_0' &= a_0 \gamma, & a_1' &= a_1 \gamma, & a_2' &= a_2 \gamma, & c' &= c \gamma, \\
b_1' &= \frac{b_1}{b_0}, & b_2' &= \frac{b_2}{b_0}, & \varepsilon' &= b_0 \varepsilon \gamma, & \delta' &= \frac{\delta}{\gamma}.
\end{align*}
$$

Without loss of generality, we can fix $b_0 = 1$ and $\gamma = 1$ in (1.1) and drop the tilde. Hence, from now on, we will consider the system

\begin{align*}
\varepsilon \delta \frac{dx}{dt} &= -y + f(x), \\
\varepsilon \frac{dy}{dt} &= a_0 x + a_1 y + a_2 + cX, \\
\varepsilon \frac{dX}{dt} &= -Y + g(X), \\
\varepsilon \frac{dY}{dt} &= X + b_1 Y + b_2.
\end{align*}

In what follows, we denote the derivation with respect to the time variable $t$ as $'= \frac{d}{dt}$. 
2.2. Qualitative behavior of the uncoupled system. The average activity of both regulating and secreting neurons is modeled by a classical FitzHugh–Nagumo system, while the control of GnRH neuron activity by regulatory neurons is expressed by means of a one-way coupling of variable $X$ onto variable $y$. Hence, we can analyze separately the slow-fast subsystem (2.2c)–(2.2d) according to the time scale parameter $\varepsilon$ and the $Y$-nullcline parameters $b_1$ and $b_2$.

The classical approach of slow-fast systems consists in studying the Boundary-Layer System obtained after fixing the slow dynamics at 0 (here $\dot{Y} = 0$) and focusing on the geometric invariants of the fast dynamics. Precisely, with the time rescaling

\begin{equation}
    t = \varepsilon \tau,
\end{equation}

subsystem (2.2c)–(2.2d) becomes

\begin{align}
    \frac{dX}{d\tau} &= -Y + g(X), \\
    \frac{dY}{d\tau} &= \varepsilon (X + b_1 Y + b_2).
\end{align}

Henceforth, system (2.4) will be denoted as $(RS_{\varepsilon})$. Posing $\varepsilon = 0$ in this last system, we obtain the corresponding Boundary-Layer System $(RS_0)$:

\begin{align}
    \frac{dX}{d\tau} &= -Y + g(X), \\
    \frac{dY}{d\tau} &= 0.
\end{align}

The system $(RS_0)$ is a zero-order approximation of $(RS_{\varepsilon})$ according to $\varepsilon$. The singular perturbation theory allows us to assess where this approximation is valid.

We consider the set of singular points of the system $(RS_0)$, defined by the cubic function $Y = g(X)$, and analyze their nature according to the fast dynamics (2.5a). The two local extrema of the cubic are nonhyperbolic points. The points on the cubic middle branch (the red branch in Figure 1) are hyperbolic and repulsive, while the left and right branches (resp., the blue and green branches in Figure 1) consist in hyperbolic attractive points. Each branch is a normally invariant hyperbolic manifold of subsystem (2.5) and, consequently, persists under perturbation by the slow dynamics (i.e., for small values of $\varepsilon$) into an $O(\varepsilon)$-close normally hyperbolic invariant manifold of $(RS_{\varepsilon})$. We recall that any orbit of $(RS_{\varepsilon})$ starting from an initial condition lying in the attraction basin of a normally attractive manifold reaches an $O(\exp(-k/\varepsilon))$-neighborhood of this manifold.

In the classical case we are interested in, the values of parameters $b_1$ and $b_2$ are small enough for the $Y$-nullcline to intersect the cubic on the middle branch at a repulsive singular point of system $(RS_{\varepsilon})$ and the two other intersection points to be far away, respectively, on the left and right branches. It is well known that, in such a case, $(RS_{\varepsilon})$ admits an attractive limit cycle surrounding the middle singular point (cf. Figure 4(a)). In the course of a complete period, the current point $(X, Y)$ first comes near, and then goes down slowly along, the left perturbed manifold (near the left branch of the cubic $Y = g(X)$) at an $O(1)$-speed. Near the
extremum of the cubic (which is a nonhyperbolic singular point of (2.5a)), it keeps going down and moves away from the cubic. At that time, it is subject to the fast motion and quickly reaches (at an $O(1/\varepsilon)$-speed) the vicinity of the right branch, as its $Y$-coordinate remains almost constant. Then it slowly goes up along the right perturbed manifold, before it finally comes back quickly near the left branch. This well-known motion is detailed in the proof of Lemma 3.1. For more details on singular perturbation theory, we refer the reader to [9] for the general study of slow-fast systems and to [1, 7, 20] for its application to oscillations.

2.3. Qualitative behavior of the whole system. We briefly recall here the specific behavior of system (2.2) and the corresponding pattern of the $y(t)$ signal generated as a model output. We refer the reader to [5] for more explanations about the model properties.

Through the coupling of (2.2a)–(2.2b) with (2.2c)–(2.2d), the Regulating System drives the dynamics of the GnRH Secreting System by controlling the position of its straight-line separatrix defined by

$$a_0x + a_1y + a_2 + cX = 0.$$ 

When all parameter values are fixed, the dynamics is determined by the value of $X$. Let us consider $X$ as a parameter. We define the branches of the cubic $\dot{x}$-nullcline $y = f(x)$ in the same manner as for $Y = g(X)$. From now on, we will denote the left branch (the pink one in Figure 2, Step 1) as $M_L$, the middle branch (the red one) as $M_m$, and the right branch (the dark green one) as $M_r$. As the value of $X$ increases in $\mathbb{R}$, the GnRH Secreting System (2.2a)–(2.2b) displays the following sequence of behaviors (see Figure 2):

1. There is a unique singular point far up on $M_L$.  
2. An inverse saddle-node bifurcation occurs on $M_r$.  
3. There are three singular points: a saddle far up on $M_L$, an attractive point on $M_r$, and another saddle on its right.
4. A supercritical Hopf bifurcation occurs as the middle singular point coincides with the local maximum of the cubic.

5. One of the three singular points lies on \( M_m \) and is surrounded by an attractive relaxation limit cycle. The two other singular points are saddles, far up on \( M_l \) and \( M_r \), respectively.

6. An inverse supercritical Hopf bifurcation occurs as the middle singular point coincides with the local minimum of the cubic; the limit cycle disappears.

7. There are three singular points: a saddle far down on \( M_r \), an attractive point on \( M_l \), and another saddle on its left. Any orbit starting near the origin reaches the vicinity of \( M_l \) and approaches the attractive singular point.

8. A saddle-node bifurcation occurs on \( M_l \).

9. There is a unique singular point far down on \( M_r \). Any orbit starting near the origin reaches the vicinity of \( M_l \) and goes up.

In what follows, we will set precise conditions on the parameters, so that, in the range of values taken by \( X \) along the limit cycle of the Regulating System, the GnRH Secreting System displays periodically the subsequence of behaviors described above from Step 5 to Step 9 and back from Step 9 to Step 5. In this case, along an orbit of (2.2), the current point \((x, y, X, Y)\) displays the following motion, illustrated by Figure 2 (Step 5 to Step 9) and the four animations (73223_01.avi [156KB], 73223_02.avi [102KB], 73223_03.avi [126KB], and 73223_04.avi [310KB]) linked to the panels of Figure 3.

When \((X, Y)\) goes down near the left branch of the cubic \( Y = g(X) \) (phase I), \( X \) increases slowly: system (2.2a)–(2.2b) displays the Step-5 behavior and \((x, y)\) turns around the limit cycle. Let us remark that the dynamics on \((x, y)\) (on the order of \( O(1/\varepsilon) \) and \( O(1/(\varepsilon \delta)) \)) is faster than the dynamics on \((X, Y)\) (on the order of \( O(1) \)), while \((X, Y)\) is near the left branch of the cubic \( Y = g(X) \). Thus, \((x, y)\) can oscillate many times before \((X, Y)\) arrives at the bottom of the left branch. Then, \( X \) increases quickly at an \( O(1/\varepsilon) \)-speed (phase II) and the limit cycle of system (2.2a)–(2.2b) disappears (Step 6). \( X \) decreases slowly (phase III), and \((x, y)\) climbs up the \( M_l \) of the cubic \( y = f(x) \) (Step 7 to Step 9). Afterward \( X \) decreases quickly at an \( O(1/\varepsilon) \)-speed (phase IV) and \((x, y)\) goes down near \( M_l \) at an \( O(1/\varepsilon) \)-speed before oscillating again around the relaxation limit cycle.

Table 1 sums up the sequence of bifurcations undergone by the GnRH Secreting System, as \((X, Y)\) describes the limit cycle, and the behaviors of the whole system. Typical behaviors of the Regulating System and the GnRH Secreting System, while they interact, are displayed in the animation (73223_05.avi [2.42MB]) linked to Figure 4. Typical orbits of system (2.2) generate \( y(t) \) signal patterns such as that represented in Figure 4(d). Hence, one can distinguish two phases in this periodic pattern: the pulsatility phase (Step-5 behavior) and the surge (Steps-6–9 behavior) displayed by the GnRH Secreting System.

2.4. Pulse amplitude. From the previous discussion, we have seen that a pulsatile \( y(t) \) signal is generated from the oscillations of \((x, y)\) around the limit cycle of the GnRH Secreting System. For all values of \( X \) leading to such oscillations, the shape of the limit cycle of system (2.2a)–(2.2b) remains almost the same (see Figure 4(c)). Consequently, the pulse amplitude is approximately equal to the difference between the \( y \)-coordinates of the two extrema \( P_f^+ \) and \( P_f^- \) of \( y = f(x) \). Thus, we can keep a unique relevant parameter to define the cubic
Figure 2. Position of the $\dot{y}$-nullcline (red straight line) and singular points of the GnRH Secreting System (2.2a)–(2.2b) as the value of $X$ increases. The panels correspond to the steps described in section 2.3. The first panel (Step 1) represents the different branches of $y = f(x)$: the left (pink) branch $M_l$, the middle (red) branch $M_m$, and the right (dark green) branch $M_r$. The uppermost (resp., lowermost) singular point lying on $M_l$ (resp., $M_r$) is not shown in Steps 1–5 (resp., Steps 4–9).
Figure 3. Animated GnRH Secreting System behavior: (a) in the middle of the pulsatility phase, (b) during the pulsatility to surge transition, (c) during the surge rise, and (d) during the surge decline. We have set $Y = \text{cst}$ ($\varepsilon$ zero-order approximation). The objects relating to the Regulating System are drawn in green, and those relating to the GnRH Secreting System are drawn in red. The green and red cubics are, respectively, the $\dot{X}$-nullcline $Y = g(X)$ and the $\dot{x}$-nullcline $y = f(x)$; the green and red straight lines are, respectively, the $\dot{Y}$-nullcline and the $\dot{y}$-nullcline; the green square represents the $(X,Y)$ current point of the Regulating System, and the red diamond represents the $(x,y)$ current point of the GnRH Secreting System; the green and red bold lines represent the $(X,Y)$ and $(x,y)$ trajectories during the associated phase. (a) $(X,Y)$ lies on the left branch of the cubic $Y = g(X)$. This configuration corresponds to the behavior of the whole system as $(X,Y)$ experiences Phase I along the Regulating System limit cycle. (b) $(X,Y)$ reaches the right branch of the cubic $Y = g(X)$ (behavior of the whole system as $(X,Y)$ experiences Phase II). (c) $(X,Y)$ lies on the right branch of the cubic $Y = g(X)$ (Phase III). (d) $(X,Y)$ reaches the left branch of the cubic $Y = g(X)$ (Phase IV). Clicking on each image displays the accompanying animation: (a) 73223_01.avi [156KB], (b) 73223_02.avi [102KB], (c) 73223_03.avi [126KB], and (d) 73223_04.avi [310KB].
Table 1

Links between the sequence of bifurcations in the GnRH Secreting System, the sequence of behaviors in the whole system (2.2), and GnRH secretion patterns. The \( \dot{y} \)-nullcline steps denoted in italics correspond to configurations in which the GnRH Secreting System remains during a short time, as \( X \) evolves quickly.

<table>
<thead>
<tr>
<th>Phase along the ((X,Y)) limit cycle</th>
<th>( \dot{y} )-nullcline position</th>
<th>Invariant manifold followed by ((x,y))</th>
<th>GnRH secretion pattern</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>5</td>
<td>Attractive limit cycle</td>
<td>Pulsatility</td>
</tr>
<tr>
<td>II</td>
<td>6-7-8</td>
<td>Climb up along ( \mathcal{M}_t )</td>
<td>Surge beginning</td>
</tr>
<tr>
<td>III</td>
<td>9</td>
<td>( \mathcal{M}_t )</td>
<td>Surge</td>
</tr>
<tr>
<td>IV</td>
<td>8-7-6</td>
<td>Descent along ( \mathcal{M}_t )</td>
<td>Surge decline</td>
</tr>
<tr>
<td>I</td>
<td>5</td>
<td>( \mathcal{M}_t ) and then attractive limit cycle</td>
<td>Surge decline</td>
</tr>
</tbody>
</table>

function \( g \) and represent the amplitude. This reparameterization is described in more detail in [5].

To simplify the calculations and keep similar expressions for \( f \) and \( g \), we pose the following:

\[
\begin{align*}
  f(x) &= -x^3 + 3\lambda^2 x, \\
  g(X) &= -X^3 + 3\mu^2 X.
\end{align*}
\]

Then, the coordinates of the local extrema, \( P_f^{\pm} \) and \( P_g^{\pm} \), as well as those of their projections on the other branch of the cubics (along the trajectories of the fast systems), \( Q_f^{\pm} \) and \( Q_g^{\pm} \), are easy to compute:

\[
\begin{align*}
  (2.6a) & \quad P_f^{+} = (\lambda, 2\lambda^3), \quad P_f^{-} = (-\lambda, -2\lambda^3), \\
  (2.6b) & \quad P_g^{+} = (\mu, 2\mu^3), \quad P_g^{-} = (-\mu, -2\mu^3), \\
  (2.7a) & \quad Q_f^{+} = (-2\lambda, 2\lambda^3), \quad Q_f^{-} = (2\lambda, -2\lambda^3), \\
  (2.7b) & \quad Q_g^{+} = (-2\mu, 2\mu^3), \quad Q_g^{-} = (2\mu, -2\mu^3).
\end{align*}
\]

These reference points and their coordinates are displayed in Figure 5.

Henceforth, we will set \( \lambda \) and \( \mu \) to given, constant values. The \( \delta \) parameter deals exclusively with the duration of the pulse maximum, since it has an effect on only the fastest motion (triple arrows in Figure 4(c)). This duration is very short compared to the interpulse interval, even when the pulse frequency is maximal, so that \( \delta \) has to be fixed to a small value. In the applications given in section 6, we assume that \( \delta = 0.0125 \), which means that the ratio
Figure 4. Typical orbit projections and signals generated by (2.2). (a) Attractive limit cycle of the Regulating System \( \text{RS}_\varepsilon \). (b) \( X(t) \) output signal from the Regulating System. (c) Projection of system (2.2) orbit onto the \((x,y)\)-plane. (d) \( y(t) \) output signal from the GnRH Secreting System. The roman numerals distinguish the pulsatility phase (I), the surge beginning (II), the surge (III), and the surge decline (IV). Single (resp., double, triple) arrows correspond to motions with an \( O(1) \) (resp., \( O(1/\varepsilon) \), \( O(1/\varepsilon^2) \)) speed. The pulsatility phase duration (resp., surge duration) corresponds, within an \( O(\varepsilon) \)-approximation, to the time during which \( X < 0 \) (resp., \( X > 0 \)) along the parameterization of the Regulating System limit cycle. Clicking on any image displays the accompanying animation (73223.05.avi [2.42MB]), representing the interacting Regulating System and GnRH Secreting System. The objects relating to the Regulating System are drawn in green, and those relating to the GnRH Secreting System are drawn in red. The green and red cubics are, respectively, the \( \dot{X} \)-nullcline \( Y = g(X) \) and the \( \dot{y} \)-nullcline \( y = f(x) \); the green and red straight lines are, respectively, the \( \dot{Y} \)-nullcline and the \( \dot{y} \)-nullcline; the green square represents the \((X,Y)\) current point of the Regulating System; and the red diamond represents the \((x,y)\) current point of the GnRH Secreting System.

between the duration of the pulse maximum and the average interpulse interval is on the order of 1 to 80. Actually, the precise value of \( \delta \) has no influence on the qualitative behavior of the model, or on the quantitative specifications considered later, as long as it is small enough for the GnRH Secreting System to admit a relaxation limit cycle in the Step-5 case described in section 2.3 and represented in Figure 2.

Each of the other parameters constitutes a degree of freedom, whose value has to be fitted to any particular list of specifications, as is illustrated in section 6. We begin in the next section by studying the effect of \( \varepsilon, b_1, \) and \( b_2 \) on the duration ratio between the surge and the whole cycle.

2.5. Control of the two-way transition between the pulsatility phase and the surge by the dynamics of the Regulating System. From the sequence of behaviors described in section 2.3, we can establish a direct link between the pattern of the GnRH secretion signal
Figure 5. Graph of the cubics defining the \( \dot{x} \) and \( X \) nullclines. The coordinates of the local extrema \( P_f^+ \) (resp., \( P_f^- \)) of the \( x \)-nullcline (resp., \( X \)-nullcline) are given in (2.6). The projections \( Q_f^+ \) (resp., \( Q_f^- \)) of these extrema along \( y = \text{cst} \) (resp., \( Y = \text{cst} \)) are given in (2.7).

and the values of \( X \) along two distinct parts of the Regulating System limit cycle.

As stated in [5], the time during which the current point \((X, Y)\) remains in a neighborhood of the right branch of \( Y = g(X) \) along the limit cycle is \( O(1) \) in \( \varepsilon \). In contrast, the transition time of the Regulating System from a neighborhood of \( P_g^+ \) to a neighborhood of \( Q_g^+ \) is \( O(\varepsilon) \). The same \( \varepsilon \)-order applies, respectively, to the slow motion near the left branch of the cubic \( Y = g(X) \) and the fast motion between \( P_g^- \) and \( Q_g^- \).

In a precise zone of the parameter space defined in section 5, we can ensure that, for \(-2\mu \leq X \leq -\mu \) (Phase I; see Figure 4), the GnRH secretion signal has a pulsatile pattern (Step 5), while, for \( \mu \leq X \leq 2\mu \) (Phase III), it switches to a surge pattern (Steps 7–9). Once \((X, Y)\) has reached the vicinity of \( Q_g^+ \) (Phase II), it takes an \( O(\varepsilon) \)-time for \((x, y)\) to reach a neighborhood of \( Q_f^- \) (Steps 8–7–6) and oscillates again around the limit cycle of the GnRH Secreting System (Step 5). Consequently, if we neglect the \( O(\varepsilon) \)-duration of the fast motions, we can approximate the duration of the pulsatility phase by the time during which \( X < 0 \) and the surge duration by the time during which \( X > 0 \) (see panels (b) and (d) of Figure 4).

3. Bifurcation diagram of the Regulating System. In this section, we first describe the sequence of bifurcations undergone by the Regulating System \((RS_\varepsilon)\), defined by (2.4), and the corresponding behaviors exhibited by the whole system (whether such behaviors are meaningful or not from the biological viewpoint) in delimited zones of the parameter space. Then, we restrict the parameter space to a domain where the whole system meets the prescribed dynamics, alternating between pulsatility phases and surges.
We are interested in determining conditions under which the Regulating System admits an attractive limit cycle, further denoted by \( C(b_1, b_2, \varepsilon) \). We have first to assume that \((RS_\varepsilon)\) admits a singular point \((X_0, Y_0 = g(X_0))\) such that \(|X_0|<\mu\), and that \(\varepsilon\) is small enough (in a sense that we will explain later in Lemma 3.1). Yet, even under those necessary conditions, two different types of bifurcation may make the limit cycle disappear: a supercritical Hopf bifurcation and a homoclinic bifurcation. To explain the occurrence of these bifurcations, we have to remember that, for any \(b_1>0\), \((RS_\varepsilon)\) admits two other singular points of saddle type \((X_-, Y_- = g(X_-)), X_- < -\mu\) and \((X_+, Y_+ = g(X_+)), X_+ > \mu\).

### 3.1. Hopf bifurcation

The Hopf bifurcation happens when the middle singular point \((X_0, Y_0)\) coincides with the local minimum of the cubic \(Y = g(X)\), i.e., when \(X_0 = -\mu\) (see Figure 2, Step 6). This condition reads

\[-\mu + b_1 g(-\mu) + b_2 = 0 \iff -\mu + b_1 \left(\mu^3 - 3\mu^3\right) + b_2 = 0.\]

Hence, the Hopf bifurcation occurs along the surface

\[\mathcal{H}_p : b_2 = h_p(b_1) = \mu + 2b_1\mu^3\]

in the parameter space \((\varepsilon, b_1, b_2)\).

The Hopf bifurcation gives birth to a small limit cycle which surrounds the middle singular point \((X_0, Y_0)\) (lying near \(P_\varepsilon^2\)) as \((\varepsilon, b_1, b_2)\) crosses \(\mathcal{H}_p\) transversally to enter the set \(b_2 < h_p(b_1)\). Since \((RS_\varepsilon)\) is a slow-fast system, this limit cycle (initially dependent on \((\varepsilon, b_1, b_2)\)) quickly becomes a big relaxation limit cycle when \((\varepsilon, b_1, b_2)\) moves away from \(\mathcal{H}_p\).

The occurrence of small limit cycles in such relaxation systems is known as the “Canard phenomenon” (see [7, 20]). The “Canard cycles” are attractive limit cycles which follow, against all expectations, a neighborhood of the fast dynamics manifold of repulsive points for a while. Some examples are displayed in Figure 6.

As explained in section 2.5, the alternation between positive and negative values of \(X\) along the Regulating System limit cycle is a necessary condition to ensure the alternation between pulsatility and surge in the GnRH secretion signal. Consequently, we make the suitable assumptions to remain in a zone of the parameter space in which the Regulating System admits a genuine limit cycle (such as the cycle shown in Figure 7(a)). We thus consider the following security plane:

\[\mathcal{H}_p^{-\alpha} : b_2 = h_p(b_1) - \alpha = \mu + 2b_1\mu^3 - \alpha, \quad \alpha > 0,\]

to stay far enough away from the Hopf bifurcation. From now on, we will consider only parameter values verifying \(b_2 < h_p(b_1) - \alpha\).

### 3.2. Relaxation limit cycle

We consider the set \(\Gamma_0\) as the expected limit-periodic set of the limit cycles, when \(\varepsilon \to 0\), for the Hausdorff distance.\(^1\) Let us define \(\Gamma_0\) as the union of the two branches of the cubic \(Y = g(X)\) linking \(Q^0_+\) to \(P^0_+\) and \(Q^0_+\) to \(P^0_+\), with the segments \([P^0_+, Q^0_+]\) and \([P^0_+, Q^0_+]\) (see Figure 7(b)):

\[\Gamma_0 = \{(X, g(X)) \mid \mu \leq |X| \leq 2\mu \} \cup [Q^0_+, P^0_+] \cup [P^0_+, Q^0_+].\]

\(^1\)The Hausdorff distance between two compacts \(K\) and \(K'\) of a metric space \((E, d)\) is the smallest \(r > 0\) such that \(\forall x \in K, \ d(x, K') < r\) and \(\forall y \in K', \ d(y, K) < r\).
Figure 6. Examples of Canard cycles of the Regulating System \((RS_\varepsilon)\) for three different, yet very close (to \(10^{-12}\)), values of \(\varepsilon\).

Figure 7. (a) Limit cycle of the Regulating System \((RS_\varepsilon)\) for the following parameter values: \(b_1 = 0.1, b_2 = 1, \varepsilon = 0.1\). (b) Limit-periodic set \(\Gamma_0\): Limit of the family of genuine limit cycles \((C(b_1, b_2, \varepsilon))_{\varepsilon \in [0, \varepsilon_0]}\) as \(\varepsilon\) tends to 0 according to the Hausdorff distance.

The following lemma gives sufficient conditions to ensure the existence of a genuine relaxation limit cycle, lying in a small (\(\varepsilon\)-dependent) neighborhood of \(\Gamma_0\).

Lemma 3.1. For each \(\alpha > 0\), for all \((b_1, b_2)\) verifying

\[
(3.3) \quad b_2 < \mu + 2b_1 \mu^3 - \alpha
\]

and

\[
(3.4) \quad b_2 < 2\mu - 2b_1 \mu^3,
\]
Figure 8. Construction of the first-return map induced by the flow from $\Sigma$ to $\Sigma$. $M_0^-$, the part of the cubic $Y = g(X)$ between $(X_-, Y_-)$ and $P_g^-$, is a normally invariant attractive manifold for the system $(RS_0)$. The part of the cubic $Y = g(X)$ between $P_g^+$ and $(X_+, Y_+)$ (not shown on the picture, far down on the right branch) is a normally invariant attractive manifold for the system $(RS_0)$. $M_0^-$ (resp., $M_0^+$) perturbs into an $O(\varepsilon)$-close manifold $M^-_\varepsilon$ (resp., $M^+_\varepsilon$), which is invariant and normally hyperbolic for $(RS_\varepsilon)$. Starting from an initial data on section $\Sigma$, transverse to $M_0^-$, we can track the orbit of $(RS_\varepsilon)$ near $M^-_\varepsilon$ until $X = -\mu$, then along a fast trajectory of $(RS_\varepsilon)$, then near $M^+_\varepsilon$ until $X = \mu$, again along a fast trajectory, and finally near $M^-_\varepsilon$ and back to $\Sigma$.

there exists $\varepsilon_0 > 0$ such that, for all $\varepsilon \in [0, \varepsilon_0[$, the limit cycle of $(RS_\varepsilon)$ exists and contains some points of $[\mu, +\infty] \times \mathbb{R}$. Moreover, for $(b_1, b_2)$ fixed, the limit cycle $C(b_1, b_2, \varepsilon)$ lies in an $O(\varepsilon^{2/3})$-neighborhood of $\Gamma_0$.

Proof. We use the sketch of the well-known proof of limit cycle existence for the FitzHugh–Nagumo system, when the left and right singular points are, respectively, far away from $P_g^-$ and $P_g^+$. This proof is based on the series expansion of the first return map induced by the flow of $(RS_\varepsilon)$. We focus here on the well-definition of this map. Based on the arguments enunciated in [1], we derive the series expansion of the map and prove the existence of one stable fixed point and that of the attractive limit cycle. The geometric objects mentioned below are represented in Figure 8.

Let $\alpha > 0$ and $(b_1, b_2)$ verify (3.3) and (3.4). The part of the cubic between $(X_-, Y_-)$ and $P_\varepsilon^-$, denoted by $M_\varepsilon ^-$, is a normally attracting manifold of the Boundary-Layer System associated to $(RS_\varepsilon)$, which perturbs into a left $O(\varepsilon)$-close normally attracting manifold of $(RS_\varepsilon)$, denoted by $M^-_\varepsilon$. Let us consider a section $\Sigma$ transverse to $M^-_\varepsilon$ of small but fixed length. We just need that the section does not intersect the middle branch of the cubic $Y = g(X)$. Then, all trajectories of $(RS_\varepsilon)$ starting from $\Sigma$ reach an exponentially small
neighborhood of \( \mathcal{M}_c^- \) until they enter an \( O(\varepsilon^{2/3}) \)-neighborhood of \( P_\varepsilon \) (see \cite{20}). According to (3.3), the middle singular point of \((RS_\varepsilon)\) is above \( P_\varepsilon \). Thus, we can choose \( \varepsilon \) small enough so that this neighborhood does not contain \((X_0, Y_0)\).

Let us remark, too, that \( \mathcal{M}_c^- \) crosses \( X = -\mu \) at a point \( P_\varepsilon \) below the point \( P_\varepsilon \), since \( \dot{y} < 0 \) along \( \mathcal{M}_c^- \). All trajectories of \((RS_\varepsilon)\) starting from an \( O(\varepsilon^{2/3}) \)-neighborhood of \( P_\varepsilon \) keep away from any singular point for \( \varepsilon \) small enough. Hence, they remain in an \( O(\varepsilon) \)-neighborhood of the fast trajectory starting from \( P_\varepsilon \), which reaches the right attractive manifold.

With the same arguments as above, we keep tracking the orbits of \((RS_\varepsilon)\) in an exponentially small neighborhood of the right \( O(\varepsilon) \)-close normally attractive manifold of \((RS_\varepsilon)\), denoted by \( \mathcal{M}_c^+ \), until they reach a point \( P_\varepsilon^+ \) lying on \( X = \mu \) and above \( P_\varepsilon^+ \). From (3.4), the left saddle point \((X_-, Y_-)\) is strictly above the point \( Q_\varepsilon^0 \) on the cubic. Thus, we can choose \( \varepsilon \) small enough to ensure that \( P_\varepsilon^+ \) lies below the stable manifold of \((X_-, Y_-)\). According to the theory of regular perturbations, the orbits which have just followed the perturbed attractive manifold remain in an \( O(\varepsilon^{2/3}) \)-neighborhood of the fast trajectory connecting \( P_\varepsilon \) with \( Q_\varepsilon^0 \).

Hence, for \( \varepsilon \) small enough, all the trajectories starting from this neighborhood cross the cubic and thereafter remain in an exponentially small neighborhood of \( \mathcal{M}_c^- \) until it crosses \( \Sigma \). Thus the first return map is well defined on \( \Sigma \) for \( \varepsilon \) small enough, i.e., \( \varepsilon \in [0, \varepsilon_0[ \). Moreover, it maps \( \Sigma \) into an exponentially small neighborhood of \( \Sigma \cap \mathcal{M}_c^- \) and is exponentially contracting. This leads to the existence of an attractive fixed point corresponding to an attractive limit cycle \( C(b_1, b_2, \varepsilon) \) lying in an \( O(\varepsilon^{2/3}) \)-neighborhood of the limit periodic set \( \Gamma_0 \) (see Figure 7) and containing some points of \([\mu, +\infty[ \times \mathbb{R} \).

Remark 3.2. The smaller \( \alpha \) is, the smaller \( \varepsilon_0 \) has to be to avoid any Canard effect. Hence, from now on, \( \alpha \) is fixed to a constant value small enough so that the region

\[
\mathcal{R}_0 = \left\{ (\varepsilon, b_1, b_2) \mid \begin{array}{l}
  b_2 < \mu + 2b_1\mu^3 - \alpha \\
  b_2 < 2\mu - 2b_1\mu^3
\end{array} \right\}
\]

is nonempty.

Moreover, the closer \( b_2 \) is from \( 2\mu - 2b_1\mu^3 \), the closer the left singular point is to the limit cycle, and the smaller \( \varepsilon_0 \) has to be. \( \varepsilon_0 \) tends to 0 as \((b_1, b_2)\) approaches \( \mathcal{H}_c^0 \).

3.3. Homoclinic bifurcation. Remark 3.2 opens the way to intuiting that the disappearance of the limit cycle through a homoclinic bifurcation occurs when the left saddle point \((X_-, Y_-)\) “crosses” the limit cycle, leading to the intersection of its stable manifold with its unstable manifold. Let us first consider \( \Gamma_0 \), the \( \varepsilon \)-zero-order approximation of the limit cycle under the conditions of Lemma 3.1. We can then develop the zero-order approximation of the homoclinic connection by considering

\[
(X_-, g(X_-)) = (X_{Q_+}, g(X_{Q_+})).
\]

It is equivalent to state that \( Q_+ = (X_{Q_+}, g(X_{Q_+})) = (-2\mu, 2\mu^3) \) lies on the separatrix \( X + b_1Y + b_2 = 0 \),

\[-2\mu + b_1g(-2\mu) + b_2 = 0,
\]

so that, in first approximation, the homoclinic bifurcation occurs near the surface:

\[
\mathcal{H}_c^0 : b_2 = h_c^0(b_1) = 2\mu - 2b_1\mu^3.
\]
Figure 9. Projections of $\mathcal{H}_p$, $\mathcal{H}_p^{-\alpha}$, and $\mathcal{H}_c^0$ onto the $\varepsilon = 0$ plane. Starting from $b_2 = 0$, one can reach $\mathcal{H}_c^0$ before $\mathcal{H}_p^{-\alpha}$ only if $(\mu + \alpha)/(4\mu^3) < b_1 < 1/\mu^2$. The inserts represent the position of the nullclines and the $\varepsilon$-zero-order approximation of the flow in each region of the parameter space except the grey one: (1) below $\mathcal{H}_p^{-\alpha}$ and $\mathcal{H}_c^0$, (2) below $\mathcal{H}_p^{-\alpha}$ and above $\mathcal{H}_c^0$, (3) above $\mathcal{H}_p$.

Now, we can seek the homoclinic connection as $b_2$ increases from 0, for $\varepsilon > 0$ and $b_1$ fixed. This connection occurs if $(\varepsilon, b_1, b_2)$ reaches the neighborhood of $\mathcal{H}_c^0$ before crossing $\mathcal{H}_p^{-\alpha}$, which amounts to the following condition on $b_1$ (see Figure 9):

$$h_c^0(b_1) = 2\mu - 2b_1\mu^3 < h_p(b_1) - \alpha = \mu + 2b_1\mu^3 - \alpha \Leftrightarrow b_1 > \frac{\mu + \alpha}{4\mu^3}.$$  

The following proposition proves the existence and gives the development of the surface of homoclinic connections in the $(\varepsilon, b_1, b_2)$ parameter space.

**Proposition 3.3.** There exists, locally near $\varepsilon = 0$, a $\mathcal{C}^1$-surface of homoclinic connections in the $(b_1, b_2, \varepsilon)$-space given by the graph $\mathcal{H}_c : b_2 = h_c(b_1, \varepsilon) = 2\mu - 2\mu^3 b_1 + O(\varepsilon^{2/3})$ defined for

$$b_1 \in \left[ \frac{\mu + \alpha}{4\mu^3}, \frac{1}{\mu^2} \right].$$

**Proof.** If condition (3.6) holds and $\varepsilon$ is fixed to a small value, then there exist $(b_1, b_2)$, verifying (3.3), (3.4), and (3.6), for which the value of $\varepsilon_0$, defined in Lemma 3.1, is greater than $\varepsilon$. The left singular point $(X_-, Y_-)$ for this value of $(b_1, b_2, \varepsilon)$ lies on the outside of an $O(\varepsilon^{2/3})$-neighborhood of $\Gamma_0$ containing the limit cycle $C(b_1, b_2, \varepsilon)$. As $b_2$ increases, the left singular point enters this neighborhood and finally crosses the limit cycle. Thus, for a value
of $b_2$ close to (and smaller than) $2\mu - 2b_1\mu^3$, the saddle undergoes a homoclinic connection where the limit cycle disappears (see Figure 10).

Let $X_{\text{min}}(b_1, b_2, \varepsilon)$ be the minimum of $X$ along the limit cycle $C(b_1, b_2, \varepsilon)$ (see Figure 7). From the fast dynamics (which is null on the cubic), we have immediately that $(X_{\text{min}}(b_1, b_2, \varepsilon), g(X_{\text{min}}(b_1, b_2, \varepsilon)))$ lies on the cubic. Consequently, the condition for the homoclinic connection reads

$$X_{\text{min}}(b_1, b_2, \varepsilon) + b_1 g(X_{\text{min}}(b_1, b_2, \varepsilon)) + b_2 = 0.$$ 

As we know that $X_{\text{min}}(b_1, b_2, \varepsilon) = X_{Q_+} + O(\varepsilon^{2/3}) = -2\mu + O(\varepsilon^{2/3})$, it also reads

$$u(b_1, b_2, \varepsilon) = -2\mu + O(\varepsilon^{2/3}) + b_1 g(-2\mu + O(\varepsilon^{2/3})) + b_2 = 0,$$

where $u$ is a $C^1$ function on its definition domain (where the limit cycle exists). For each $b_1$ value such that

$$(3.8) \quad b_1 \in \left[ \frac{\mu + \alpha}{4\mu^3}, \frac{1}{\mu^2} \right],$$

$X_{\text{min}}(b_1, 2\mu - 2b_1\mu^3, 0)$ is well defined (it is equal to $X_{Q_+}$) and

$$\frac{\partial u}{\partial b_2}(b_1, 2\mu - 2b_1\mu^3, 0) = 1.$$ 

Then, the implicit function theorem implies the existence of a unique root of $u$ with respect to $b_2$ for any $b_1$ verifying (3.8) and $\varepsilon$ small enough. From (3.7), this root can be expanded as

$$b_2 = 2\mu - 2\mu^3 b_1 + O(\varepsilon^{2/3}), \quad \varepsilon \to 0.$$
3.4. Parameter space reduction. From the bifurcation diagram, we can reduce the parameter space in order to impose a periodic behavior to the Regulating System. We can also ensure that, along its attractive periodic orbit, the $X$ variable alternatively takes negative and positive values. Since the sign of $X$ along the orbit of $(RS_\varepsilon)$ discriminates between the two phases (pulsatility phase and surge) of the whole system (2.2), the restriction of the value domain for $(\varepsilon, b_1, b_2)$ is the first step in obtaining an alternation of pulsatility phases and surges.

In what follows, we will consider only $(\varepsilon, b_1, b_2)$ to be below both the surface $\mathcal{H}_c$ of homoclinic connections and the security plane $\mathcal{H}_p^{-\alpha}$ on the underside of the Hopf bifurcations plane. Hence we assume that

$$
(b_1, b_2, \varepsilon) \in \mathcal{R}_1 = \left\{(b_1, b_2, \varepsilon) \in \mathbb{R}_3^+ \middle| \begin{array}{c}
b_2 < h_p(b_1) - \alpha \\
b_2 < h_c(b_1, \varepsilon) \\
\varepsilon < \varepsilon_0
\end{array} \right\},
$$

where $\varepsilon_0$ is defined in Lemma 3.1 (see Figure 11).

![Figure 11. Bifurcation diagram for $\varepsilon < \varepsilon_0$. We restrict the parameter space to $\mathcal{R}_1$: Below the surface of homoclinic bifurcations $\mathcal{H}_c$ and far from the surface of Hopf bifurcations $\mathcal{H}_p$. In $\mathcal{R}_1$, we are assured that the Regulating System admits an attractive limit cycle and that, along this orbit, $X$ takes alternatively positive and negative values. Both conditions are necessary to obtain the alternation of surges and pulsatility phases for the $y(t)$ signal generated by the whole system (2.2).](image)

4. Foliation of the Regulating System parameter space. In this section, we focus on the way to fulfill the specification prescribing a given ratio between the pulsatility phase and the surge durations. First, we define these durations precisely according to the sign of $X$ along the Regulating System limit cycle. Then, we prove the possibility of reaching any given ratio by choosing the value of $(\varepsilon, b_1, b_2)$ in $\mathcal{R}_1$.

4.1. Pulsatility phase and surge durations. As we have seen in section 2.5, these durations are related to the time during which the current point $(X, Y)$ lies either in the half-plane...
Their ratio remains unchanged after time rescaling.

\[ T \leq 0 \ (	ext{pulsatility phase}) \text{ or } T > 0 \ (	ext{surge}) \] within one period along the limit cycle of \((RS_\varepsilon)\).

Their ratio remains unchanged after time rescaling.

**Lemma 4.1.** For \((b_1, b_2, \varepsilon) \in \mathcal{R}_1\), let us consider a parameterization \((X(t), Y(t))\) of the limit cycle \(C(b_1, b_2, \varepsilon)\) such that \(X(0) = 0\) and \(Y(0) > 0\), and let \(T(b_1, b_2, \varepsilon)\) be its period. Then, there exists a unique \(\Psi\) where
\[
(4.1a) \quad T_-(b_1, b_2, \varepsilon) = T(b_1, b_2, \varepsilon) - T_-(b_1, b_2, \varepsilon). \tag{4.1a}
\]

\[
(4.1b) \quad T_+(b_1, b_2, \varepsilon) = \int_{X_{\min}(b_1, b_2, \varepsilon)}^{X_{\max}(b_1, b_2, \varepsilon)} \frac{g'(X) + \frac{\partial \Psi}{\partial X}(X, \varepsilon)X + b_1(g(X) + \Psi_-(X, \varepsilon)) + b_2}{X + b_1(g(X) + \Psi_+(X, \varepsilon)) + b_2} dX + O(\varepsilon), \tag{4.1b}
\]

where \(\Psi_- < 0\) and \(\Psi_+ > 0\) are differentiable functions defined respectively on
\[
[X_{\min}(b_1, b_2, \varepsilon), -\mu] \times [0, \varepsilon_0]
\]

and
\[
[\mu, X_{\max}(b_1, b_2, \varepsilon)] \times [0, \varepsilon_0]
\]

such that
\[
(4.2) \quad \exists \lambda(\varepsilon) = O(\varepsilon^{2/3}), \left\{ \begin{array}{l} \forall X \in [X_{\min}(b_1, b_2, \varepsilon), -\mu], |\Psi_-(X, \varepsilon)| < \lambda(\varepsilon), \\ \forall X \in [\mu, X_{\max}(b_1, b_2, \varepsilon)], |\Psi_+(X, \varepsilon)| < \lambda(\varepsilon). \end{array} \right. \tag{4.2}
\]

**Proof.** We consider only the left part of the limit cycle, along which \(X < 0\), and prove the existence of \(\Psi_-\). The argument for the right part is identical.

Let us assume that \((b_1, b_2, \varepsilon) \in \mathcal{R}_1\) and consider the set
\[
(4.3) \quad U = \{(X, Y) \mid X < -\mu, Y < g(X_{\min}(b_1, b_2, \varepsilon)), Y < g(X)\}
\]

represented in Figure 12. Since \(\dot{X} = 0\) along the left branch of the critical manifold and \(\dot{Y} < 0\) in \(U\), any orbit of \((RS_\varepsilon)\) starting in \(U\) escapes from \(U\) across the half-line \(\{(X, Y) \mid X = -\mu, Y < -2\mu^3\}\). From the dynamics of \((RS_\varepsilon)\), we have also, along any trajectory remaining in \(U\),
\[
(4.4) \quad \frac{dY}{dX} = \frac{\dot{Y}}{\dot{X}} = \varepsilon X + b_1 Y + b_2 - Y + g(X) < 0. \tag{4.4}
\]

It follows from (4.4) that such a trajectory can be represented as the graph of a differentiable function of \(X\). In particular, the limit cycle \(C(b_1, b_2, \varepsilon)\) goes through the point \((X_{\min}(b_1, b_2, \varepsilon), g(X_{\min}(b_1, b_2, \varepsilon)))\), enters \(U\), and remains in \(U\) until it crosses the half-line \(\{(X, Y) \mid X = -\mu, Y < -2\mu^3\}\). Let us represent this trajectory as
\[
(4.5) \quad \tilde{C}_-(b_1, b_2, \varepsilon) : Y = \gamma_{b_1, b_2, \varepsilon}(X), \quad X \in [X_{\min}(b_1, b_2, \varepsilon), -\mu]
\]
and pose
\[
\Psi_-(X, \varepsilon) \to g_{b_1, b_2, \varepsilon}(X) - g(X).
\]

We can directly deduce that \(\Psi_- < 0\) and is differentiable with respect to \(X\). Since \((b_1, b_2, \varepsilon)\) lies in \(R_1\), the one-parameter family \((C(b_1, b_2, \varepsilon))_{\varepsilon \in [0, \varepsilon_0]}\) converges to the limit periodic set \(\Gamma_0\) described in Lemma 3.1 and each \(\tilde{C}_-(b_1, b_2, \varepsilon)\) trajectory, \(\varepsilon \in [0, \varepsilon_0]\), remains in an \(O(\varepsilon^{2/3})\)-neighborhood of \(\{(X, g(X)) \mid X \in [X_{\min}(b_1, b_2, \varepsilon), -\mu]\}\). Thus, \(\Psi_-\) is differentiable on \([X_{\min}(b_1, b_2, \varepsilon), -\mu] \times [0, \varepsilon_0]\) and verifies (4.2).

By definition of \(\Psi_-\), the open part of the limit cycle from \(X = X_{\min}(b_1, b_2, \varepsilon)\) to \(X = -\mu\), i.e., \(\tilde{C}_-(b_1, b_2, \varepsilon)\), is defined by
\[
Y = g(X) - \Psi_-(X, \varepsilon), \quad X \in [X_{\min}(b_1, b_2, \varepsilon), -\mu].
\]

Using the \(Y\) dynamics (2.2d), we obtain that the time taken to go along \(\tilde{C}_-(b_1, b_2, \varepsilon)\) is given by
\[
\int_{\tilde{C}_-(b_1, b_2, \varepsilon)} dt = \int_{\tilde{C}_-(b_1, b_2, \varepsilon)} \frac{dY}{X + b_1 Y + b_2} = \int_{X_{\min}(b_1, b_2, \varepsilon)}^{\mu} \frac{g'(X) + \frac{\partial \Psi_-(X, \varepsilon)}{\partial X}(X, \varepsilon) + b_2}{X + b_1 g(X) + \Psi_-(X, \varepsilon) + b_2} dX.
\]

Moreover, by application of the regular perturbation theory, the times taken to go, along the limit cycle \(C(b_1, b_2, \varepsilon)\), from \(X = 0\) to \(X = X_{\min}(b_1, b_2, \varepsilon)\) (as \(Y > 0\)) and from \(X = -\mu\) to \(X = 0\) (as \(Y < 0\)) are \(O(\varepsilon)\). Finally, the time during which \(X\) remains negative along one
period of the limit cycle is given by (4.1a). With the same construction, we obtain that the
time during which \( X \) remains positive along one period of the limit cycle is given by (4.1b),
which completes the proof. 

It is worth noticing that, as \( \varepsilon \) tends to 0, the durations \( T_-(b_1, b_2, \varepsilon) \)
and \( T_+(b_1, b_2, \varepsilon) \) tend to the limit durations

\[
T_0^-(b_1, b_2) = \int_{-\mu}^{-\mu} \frac{g'(X)}{X + b_1 g(X) + b_2} dX,
\]
\[
T_0^+(b_1, b_2) = \int_{\mu}^{\mu} \frac{g'(X)}{X + b_1 g(X) + b_2} dX,
\]

which were chosen as approximations to compute the period of the limit cycle in [5]. When
\( (b_1, b_2, \varepsilon) \) is far under the surface of homoclinic bifurcations \( \mathcal{H}_c \), it is indeed a good approximation
(with a controlled \( O(\varepsilon^{2/3}) \)-error). But, in our case, since the duration of the pulsatility
phase has to be much longer than the surge duration, we have to increase \( T_-(b_1, b_2, \varepsilon) \) in
comparison with \( T_+(b_1, b_2, \varepsilon) \). To do so, the current point \((X, Y)\) has to be confined for a
while in the vicinity of the left singular point, where \((b_1, b_2, \varepsilon)\) may be very close to \( \mathcal{H}_c \). This
is why we cannot neglect the time spent along the path from \( X = X_{\min} \) to \( X = -2\mu \), even
more so since the motion is very slow near the singular point.

### 4.2. Dependence of the approximate pulsatility phase and surge durations on \((b_1, b_2)\).

We first consider the case \( \varepsilon = 0 \) and state some needed results about the dependence of \( T_0^- \)
and \( T_0^+ \) on \( b_1 \) and \( b_2 \).

**Lemma 4.2.** (1) For all \( b_1 \in [0, 1/\mu^2] \), \( b_2 \to T_0^-(b_1, b_2) \) \((\text{resp.}, \ b_2 \to T_0^+(b_1, b_2))\) is a \( C^1 \)
strictly increasing \((\text{resp.}, \ strictly \ decreasing)\) function on

\[
A^{b_1} = \left[ 0, \min \left( h_p(b_1) - \alpha, h_p^0(b_1) \right) \right].
\]

(2)

\[
\forall b_1 \in \left[ \frac{\mu + \alpha}{4\mu^3}, \frac{1}{\mu^2} \right], \quad \lim_{b_2 \to -2\mu} T_0^-(b_1, b_2) = +\infty.
\]

In \( \mathcal{R}_1 \), \( T_0^+(b_1, b_2) \) remains finite.

(3) The function \( b_1 \to T_0^-(b_1, h_p(b_1) - \alpha) \) \((\text{resp.}, \ b_1 \to T_0^+(b_1, h_p(b_1) - \alpha))\) is \( C^1 \) and strictly
increasing \((\text{resp.}, \ strictly \ decreasing)\) on \( B = [0, 4\mu^3/\mu]. \) Moreover,

\[
\lim_{b_2 \to -2\mu} T_0^-(b_1, h_p(b_1) - \alpha) = +\infty.
\]

**Proof.** In what follows, we denote \( I_- = [-2\mu, -\mu] \) and \( I_+ = [\mu, 2\mu] \). From (4.5) and (4.6),
we can see that \( T_0^-(b_1, b_2) \) and \( T_0^+(b_1, b_2) \) are continuous functions in \( \mathcal{R}_1 \cap \{ \varepsilon = 0 \} \).

(1) Let us pose

\[
\varphi_{b_1} : (b_2, X) \to \frac{g'(X)}{X + b_1 g(X) + b_2}.
\]

Let \( b_1 \in [0, 1/\mu^2] \) fixed. We denote \( A_K = [0, K] \). Then, for all \( K \in A^{b_1} \), for all \( X \in I_- \)
\((\text{resp.}, \ I_+)\), \( \min_{b_2 \in A_K} |X + b_1 g(X) + b_2| \) is well defined and strictly negative \((\text{resp.}, \ positive)\)
for \( X \in I_- \) \((\text{resp.}, \ X \in I_+)\). Thus, the following hold:
Thus, for all $b_2 \in A^{b_1}$, $X \rightarrow \varphi_{b_1}(b_2, X)$ is a $C^1$ and integrable function on $I_-$ (resp., $I_+$).

- The function
  
  $$
  (b_2, X) \rightarrow \frac{\partial \varphi_{b_1}(b_2, X)}{\partial b_2} = -\frac{g'(X)}{(X + b_1 g(X) + b_2)^2}
  $$

  is continuous on $A^{b_1} \times I_-$ (resp., $A^{b_1} \times I_+$).

- For all $K \in A^{b_1}$, for all $b_2 \in A_K$, and for all $X \in I_-$ (resp., $I_+$),
  
  $$
  \left| \frac{\partial \varphi_{b_1}(b_2, X)}{\partial b_2} \right| \leq \frac{|g'(X)|}{\left[ \min_{b_2 \in A_K} (X + b_1 g(X) + b_2) \right]^2} = \zeta_{b_1}^{b_2}(X) < +\infty.
  $$

- For all $K \in A$, $\zeta_{b_1}^{b_2}$ is an integrable function on $I_-$ (resp., $I_+$).

Thus, from the theorem of differentiation under the integral sign, the functions

$$
 b_2 \rightarrow \int_{-2\mu}^{-\mu} \varphi_{b_1}(b_2, X) dX \quad \text{and} \quad b_2 \rightarrow \int_{2\mu}^{\mu} \varphi_{b_1}(b_2, X) dX
$$

are $C^1$ on all the $A_K$, $K \in A^{b_1}$, so that they are $C^1$ on $A^{b_1}$, and

$$
\frac{\partial}{\partial b_2} \int_{-2\mu}^{-\mu} \varphi_{b_1}(b_2, X) dX = \int_{-2\mu}^{-\mu} \frac{\partial \varphi_{b_1}(b_2, X)}{\partial b_2} dX = -\int_{-2\mu}^{-\mu} \frac{g'(X)}{(X + b_1 g(X) + b_2)^2} dX,

\frac{\partial}{\partial b_2} \int_{2\mu}^{\mu} \varphi_{b_1}(b_2, X) dX = \int_{2\mu}^{\mu} \frac{\partial \varphi_{b_1}(b_2, X)}{\partial b_2} dX = -\int_{2\mu}^{\mu} \frac{g'(X)}{(X + b_1 g(X) + b_2)^2} dX.
$$

Finally, as $g' < 0$ on $I_-$ and $I_+$, one obtains that $b_2 \rightarrow T_0^b(b_1, b_2)$ is a $C^1$ strictly increasing function on $A^{b_1}$ and $b_2 \rightarrow T_+^b(b_1, b_2)$ is a $C^1$ strictly decreasing function on $A^{b_1}$.

(2) Let $b_1 \in [\frac{\mu + \alpha}{4\mu^3}, \frac{1}{\mu}]$. The limit comes directly from the divergent integral

$$
\int_{-2\mu}^{-\mu} \frac{g'(X)}{X + b_1 g(X) + b_2} dX
$$

as $b_2$ tends to $2\mu - 2b_1 \mu^3$. On the other hand, $b_2 \rightarrow T_+^b(b_1, b_2)$ is a $C^1$ positive strictly decreasing function, so that it tends to a finite limit as $b_2$ tends to $2\mu - 2b_1 \mu^3$.

(3) Let us denote $B_K = [0, K]$ and

$$
\varphi_{H_p^{-\alpha}} : (b_1, X) \rightarrow \frac{g'(X)}{X + b_1 g(X) + \mu + 2b_1 \mu^3 - \alpha}.
$$

Let us recall that

$$
0 \leq b_1 < \frac{\mu + \alpha}{4\mu^3} \quad \text{and} \quad b_2 = \mu + 2b_1 \mu^3 - \alpha \implies X + b_1 g(X) + \mu + 2b_1 \mu^3 - \alpha < 0.
$$

Thus, for all $K \in B$, $\min_{b_1 \in B_K} |X + b_1 g(X) + \mu + 2b_1 \mu^3 - \alpha|$ is well defined and strictly negative (resp., positive) for $X \in I_-$ (resp., $X \in I_+$). Then the following hold:

- For all $b_1 \in B$, $X \rightarrow \varphi_{H_p^{-\alpha}}(b_1, X)$ is a $C^1$ and integrable function on $I_-$ (resp., $I_+$).
The function
\[
(b_1, X) \to \frac{\partial \varphi}{\partial b_1}(b_1, X) = -\frac{g'(X)(g(X) + 2\mu^3)}{(X + b_1g(X) + \mu + 2b_1\mu^3 - \alpha)^2}
\]
is continuous on \(B \times I_-\) (resp., \(B \times I_+\)).

For all \(K \in [0, \frac{\mu + \alpha}{4\mu^3}]\), for all \(b_1 \in B_K\), and for all \(X \in I_-\) (resp., \(I_+\)),
\[
\left| \frac{\partial \varphi_{H_{\mu}^\alpha}}{\partial b_1}(b_1, X) \right| \leq \frac{|g'(X)|(g(X) + 2\mu^3)}{\left[ \min_{b_1 \in B_K} |X + b_1g(X) + \mu + 2b_1\mu^3 - \alpha| \right]^2} = \varphi_{H_{\mu}^\alpha}(X) < +\infty.
\]

For all \(K \in [0, \frac{\mu + \alpha}{4\mu^3}]\), \(\varphi_{H_{\mu}^\alpha}\) is an integrable function on \(I_-\) (resp., \(I_+\)).

From the theorem of differentiation under the integral sign, the functions
\[
b_1 \to \int_{-2\mu}^{-\mu} \varphi_{H_{\mu}^\alpha}(b_1, X)dX \quad \text{and} \quad b_1 \to \int_{2\mu}^{\mu} \varphi_{H_{\mu}^\alpha}(b_1, X)dX
\]
are \(C^1\) on all the \(B_K\), \(K \in B\); thus it is \(C^1\) on \(B\), and
\[
\frac{\partial}{\partial b_1} \int_{-2\mu}^{-\mu} \varphi_{H_{\mu}^\alpha}(b_1, X)dX = \int_{-2\mu}^{-\mu} \frac{\partial \varphi_{H_{\mu}^\alpha}}{\partial b_1}(b_1, X)dX = -\int_{-2\mu}^{-\mu} \frac{g'(X)(g(X) + 2\mu^3)}{(X + b_1g(X) + \mu + 2b_1\mu^3 - \alpha)^2}dX,
\]
\[
\frac{\partial}{\partial b_1} \int_{2\mu}^{\mu} \varphi_{H_{\mu}^\alpha}(b_1, X)dX = \int_{2\mu}^{\mu} \frac{\partial \varphi_{H_{\mu}^\alpha}}{\partial b_1}(b_1, X)dX = -\int_{2\mu}^{\mu} \frac{g'(X)(g(X) + 2\mu^3)}{(X + b_1g(X) + \mu + 2b_1\mu^3 - \alpha)^2}dX.
\]

As \(g' < 0\) and \(g > -2\mu^3\) on \(\tilde{I}_-\) and \(\tilde{I}_+\), one finally obtains that \(b_1 \to T^0_\mu(b_1, h_\mu(b_1) - \alpha)\) (resp., \(b_1 \to T^0_\mu(b_1, h_\mu(b_1) - \alpha)\)) is \(C^1\) strictly increasing (resp., decreasing) on \(B\).

4.3. Control of the duration ratio. We now explain how to select the value of \(b_2\) from fixed values of \(b_1\) and \(\varepsilon\) to meet any constraint on the ratio \(T_-/T_+\).

Proposition 4.3. There exists a \(C^1\)-foliation of \(\mathcal{R}_1 \cap \{\varepsilon = 0\}\) of one-dimensional leaves such that

1. for each leaf \(\mathcal{F}_r\), there exists \(b_1^r \in [0, \frac{\mu + \alpha}{4\mu^3}]\) such that \(\mathcal{F}_r\) is the graph
\[
b_2 = l^0_r(b_1), \quad b_1 \in \left[ b_1^r, \frac{1}{\mu^2} \right]
\]
of a differentiable function \(l^0_r\);

2. for each \(r \geq 1\), there is a unique leaf \(\mathcal{F}_r^0\) on which
\[
\frac{T^0_\mu(b_1, b_2)}{T_\mu^0(b_1, b_2)} = r.
\]
Figure 13. Leaves $F^0_r$, $r = 2, 3, 6, 9, 12$, of the foliation of $R_1 \cap \{ \varepsilon = 0 \}$ defined in Proposition 4.3. Each leaf $F^0_r$, $r > 1$, is defined by $T^0_-(b_1, b_2) = r$ and given by a graph $b_2 = l^0_r(b_1)$ above $[b_1', 1/\mu^2]$. For each $r > 1$, $b_1'$ is defined by the unique $(b_1', b_2') \in L$ (in fuchsia) such that $T^0_-(b_1', b_2')/T^0_+(b_1', b_2') = r$.

Proof. Let us notice that for any value of $b_1$,

$$\frac{T^0_-(b_1, 0)}{T^0_+(b_1, 0)} = 1.$$ 

This comes from the central symmetry of the dynamics with respect to the origin when $b_2 = 0$.

From Lemma 4.2(1), for any fixed value of $b_1$, $T^0_-(b_1, b_2)$ increases and $T^0_+(b_1, b_2)$ decreases as $b_2$ increases. Thus,

$$(4.8) \quad \frac{\partial T^0_-(b_1, b_2)}{\partial b_2} T^0_+(b_1, b_2) - \frac{\partial T^0_+(b_1, b_2)}{\partial b_2} T^0_-(b_1, b_2) > 0.$$ 

Hence, $T^0_+/T^0_-$ increases as $b_2$ increases.

Let us first consider $b_1$ fixed such that

$$\frac{\alpha + \mu}{4\mu^3} \leq b_1 < \frac{1}{\mu^2}.$$ 

We can increase $b_2$ until $(b_1, b_2)$ reaches the line $H_0$. From Lemma 4.2(2), when $b_2$ tends to $2\mu - 2\mu^3 b_1$, $T^0_-(b_1, b_2)$ tends to $+\infty$, whereas $T^0_+(b_1, b_2)$ remains finite. Together with (4.8), this proves that, for each $r \geq 1$, there exists a unique solution $b_2 = l^0_r(b_1)$ in $R_1 \cap \{ \varepsilon = 0 \}$ of (4.7). As $T^0_\pm(b_1, b_2)$ are $C^1$-functions with respect to $(b_1, b_2)$, the function $l^0_r$ is $C^1$ with respect to $b_1$.

On the other hand, let us consider (see Figure 13)

$$(4.9) \quad L = \{(0, b_2) \mid 0 \leq b_2 \leq h_p(0) - \alpha\} \cup \{(b_1, b_2) \mid 0 \leq b_1 < \frac{\mu + \alpha}{4\mu^3}, b_2 = h_p(b_1) - \alpha\}.$$
We now prove, for each $r \geq 1$, the existence and unicity of $(\tilde{b}_1^r, \tilde{b}_2^r) \in \mathcal{L}$ such that

$$\frac{T^0(\tilde{b}_1^r, \tilde{b}_2^r)}{T^0_+(\tilde{b}_1^r, \tilde{b}_2^r)} = r.$$ 

From Lemma 4.2(1), for $b_1 = 0$, as $b_2$ increases from $0$ to $h_p(b_1) - \alpha$, $T^0_0/T^0_+$ is strictly increasing from $1$ to

$$(4.10) \quad \frac{T^0(0, h_p(0) - \alpha)}{T^0_+(0, h_p(0) - \alpha)}.$$

This proves the result for $r$ lower than $(4.10)$ with $\tilde{b}_1^r = 0$ and $0 \leq \tilde{b}_2^r \leq h_p(0) - \alpha$.

Now, from Lemma 4.2(3), for all $b_1 \in B$,

$$\frac{\partial}{\partial b_1} \left( \frac{T^0_0(b_1, h_p(b_1) - \alpha)}{T^0_+(b_1, h_p(b_1) - \alpha)} \right) = \frac{1}{\left( \int_{-2\mu}^{\mu} \varphi_{H_p}^{-\alpha}(b_1, X) dX \right)^2} \left[ \int_{-2\mu}^{\mu} \varphi_{H_p}^{-\alpha}(b_1, X) dX \int_{2\mu}^{\mu} \varphi_{H_p}^{-\alpha}(b_1, X) dX - \int_{-2\mu}^{\mu} \varphi_{H_p}^{-\alpha}(b_1, X) dX \int_{2\mu}^{\mu} \frac{\partial \varphi_{H_p}^{-\alpha}(b_1, X)}{\partial b_1} dX \right] > 0.$$

Consequently, as $b_1$ increases from $0$ to $(\mu + \alpha)/(4\mu^3)$, the ratio $T^0_0/T^0_+$ along $H_p^{-\alpha}$ is strictly increasing from the value $(4.10)$ to $+\infty$.

Finally, each leaf $b_2 = l_\nu(b_1)$ is defined as a graph over $[\tilde{b}_1^r, 1/\mu^2]$, where

$$\tilde{b}_1^r = 0 \quad \text{if} \quad r \leq T^0_0(0, h_p(0) - \alpha)/T^0_+(0, h_p(0) - \alpha),$$

$$\tilde{b}_1^r \in \left[ 0, \frac{\mu + \alpha}{4\mu^3} \right] \quad \text{if} \quad r > T^0_0(0, h_p(0) - \alpha)/T^0_+(0, h_p(0) - \alpha),$$

and, if we fix $r > 1$, for each $b_1$ such as

$$\tilde{b}_1^r \leq b_1 \leq \frac{\alpha + \mu}{4\mu^3},$$

there exists a unique solution $b_2 = l_\nu(b_1)$ of $(4.7)$. \hfill \blacksquare

**Theorem 4.4.** There exists a $\mathcal{C}^1$-foliation of $R_1$, defined near $\varepsilon = 0$, of two-dimensional leaves such that

1. each leaf is the graph

$$b_2 = l_r(b_1, \varepsilon), \quad b_1 \in \left[ \tilde{b}_1^r, \frac{1}{\mu^2} \right],$$

of a differentiable function $l_r$;

2. for each $r \geq 1$, there is a unique leaf $F_r$ on which

$$\frac{T_-(b_1, b_2, \varepsilon)}{T_+(b_1, b_2, \varepsilon)} = r.$$
Proof. We prolong $T_{\pm}(b_1, b_2, \varepsilon) = T^0_{\pm}(b_1, b_2)$ into a differentiable function at $\varepsilon = 0$ by posing $T_{\pm}(b_1, b_2, 0) = T^0_{\pm}(b_1, b_2)$. Let us fix $r > 1$ and $\bar{b}_1$ such that $\bar{b}_1' < \bar{b}_1 < \frac{1}{\mu^2}$. Then, like in the previous proof,

$$\frac{\partial T_-}{\partial b_2}(\bar{b}_1, l^0_r(b_1), 0) T_+(\bar{b}_1, l^0_r(b_1), 0) - \frac{\partial T_+}{\partial b_2}(\bar{b}_1, l^0_r(b_1), 0) T_- (\bar{b}_1, l^0_r(b_1), 0) > 0.$$ 

The implicit function theorem implies that there exist an open subset $U_{\bar{b}_1}^r$ in the space $\{(\varepsilon, b_1) \mid \varepsilon \geq 0\}$ which contains $(0, \bar{b}_1)$ and a differentiable function $l_r$ such that, for each $(\varepsilon, b_1)$ in $U_{\bar{b}_1}^r$,

$$\frac{T_-(b_1, b_2, \varepsilon)}{T_+(b_1, b_2, \varepsilon)} = r$$

admits a unique solution defined by

$$b_2 = l_r(b_1, \varepsilon).$$

By recovering all values of $\bar{b}_1$ such that $\bar{b}_1' < \bar{b}_1 < \frac{1}{\mu^2}$, we define $l_r$ for $b_1$ in $[\bar{b}_1, \frac{1}{\mu^2}]$ and $\varepsilon_0$ small enough. 

We have thus proved that, for any prescribed ratio $r$ of the duration of the pulsatility phase to the surge duration, there exists a two-dimensional manifold of solutions in the parameter space $(\varepsilon, b_1, b_2)$, and we have provided an $O(\varepsilon^{2/3})$ approximation of the leaf by the surface:

$$\left\{ (\varepsilon, b_1, b_2) \mid 0 \leq \varepsilon \leq \varepsilon_0, b_2 = l^0_r(b_1), \bar{b}_1' \leq b_1 \leq \frac{1}{\mu^2} \right\}.$$ 

Figure 14. Leaves in $(\varepsilon, b_1, b_2)$-space defined by $T_-/T_+ = r$ for $r = 2, 3, 6, 9, 12$. This ratio corresponds to the pulsatility phase duration over the surge duration.

We can find numerically the relevant surface solutions for different ratios, as is illustrated in Figure 14 for $r = 2, 3, 6, 9, 12$. The higher $r$ is, the closer the leaf is to the surface of homoclinic connections.
5. Tuning of the parameters of the GnRH Secreting System. In this section, we describe the process for tuning the values of $\varepsilon$, $a_0$, $a_1$, $a_2$, and $c$ with respect to a definite list of quantitative specifications. We first state the necessary conditions to ensure that system (2.2) displays the alternation between pulsatility and surge described in section 2.3. Using geometric tools, we then analyze the effect of each parameter on each prescribed ratio (duration of the pulsatility phase over the surge duration, pulse amplitude over surge amplitude, and increase in pulse frequency from the luteal to the follicular phase). Finally, we describe an algorithmic procedure to apply in order to tune the parameter values one after another and fulfill the specifications.

5.1. Constraints on the parameters to keep the right order in the sequence of secretion patterns. We recall the geometric fact that, for a fixed value of $X$, $\dot{y} > 0$ (resp., $\dot{y} < 0$) on the right (resp., left) of the $\dot{y}$-nullcline in the $(x, y)$ plane.

We first study the parameter conditions under which the $y(t)$ signal does start to pulse right after the surge. We have to prevent the GnRH Secreting System from exploring the Steps-1–4 behaviors described in section 2.3, wherever $(X,Y)$ may be along the limit cycle of the Regulating System. In other words, the Hopf bifurcation (Step 4) of the GnRH Secreting System has to occur for a value of $X$ such that $X < -2\mu$. This condition amounts to saying that, for $X = -2\mu$, $\dot{y}$ is positive at the extremum $P^f_+$ (see Figure 15), so that we have to impose the following inequality on $c$:

$$c \leq \frac{a_0 \lambda + 2a_1 \lambda^3 + a_2}{2\mu}.$$  

(Figure 15. Constraint 1: The GnRH Secreting System must not explore the Step 1-to-4 behaviors (described in section 2.3 and Figure 2) wherever $(X,Y)$ may be along the limit cycle of the Regulating System. For $X = -2\mu$ (approximate value of $X_{\text{min}}(b_1, b_2, \varepsilon)$, left panel), the $\dot{y}$-nullcline must lie in the closed nonhatched half-plane (right panel). Hence, we assume that, for $X = -2\mu$, the extremum $P^f_+$ lies on the right of the $\dot{y}$-nullcline, i.e., $\dot{y} > 0$ at $P^f_+$.

We also have to ensure that the pulsatility phase does not break off before the Regulating System switches to the fast motion from $P^s_-$ to $Q^s_-$ that induces the surge. The GnRH
Secreting System has to keep oscillating long enough that the Step 6 reverse Hopf bifurcation cannot occur for values of $X$ strictly smaller than $-\mu$. Hence, we assume that, for $X = -\mu$, $\dot{y}$ is negative at the extremum $P^f_+$ (see Figure 16). This condition reads

$$c \geq \frac{-a_0 \lambda - 2a_1 \lambda^3 + a_2}{\mu}.$$  

(5.2)

Figure 16. Constraint 2: The GnRH Secreting System must remain in the pulsatility phase (Step-5 behavior) until the Regulating System switches to the fast motion from $P^g_+$ to $Q^g_+$. For $X = -\mu$ (left panel), the $\dot{y}$-nullcline must lie in the closed nonhatched half-plane (right panel). Hence, we have to impose that the extremum $P^f_+$ lies on the left of the $\dot{y}$-nullcline, i.e., for $X = -\mu$, $\dot{y} < 0$ at $P^f_+$.

The GnRH Secreting System has to remain in the surge mode (when $P^f_+$ lies on the right of the $\dot{y}$-nullcline; see Figure 17) until $X$ undergoes the fast motion between $P^g_+$ and $Q^g_+$ that brings the GnRH Secreting System back to the pulsatile mode. This again imposes a condition on the reverse Hopf bifurcation that should not occur for a value of $X$ strictly greater than $\mu$:

$$c \geq \frac{a_0 \lambda + 2a_1 \lambda^3 - a_2}{\mu}.$$  

(5.3)

Bringing together the last two conditions, we obtain the following constraint:

$$c \geq \left| \frac{a_0 \lambda + 2a_1 \lambda^3 - a_2}{\mu} \right|.$$  

(5.4)

Finally, we have to take care that the current point $(x, y)$ does cross the $\dot{y}$-nullcline from the left to the right when $(X, Y)$ undergoes the fast motion from $P^g_+$ to $Q^g_+$. To this end, the slope of the $\dot{y}$-nullcline ($a_0/a_1$) has to be steep enough; hence the value of $a_1$ has to be small enough. Otherwise the current point $(x, y)$ might escape from control after the surge and continue climbing up the left branch of the cubic $y = f(x)$, on the right side of the $\dot{y}$-nullcline (i.e., above the uppermost left singular point in Step-5 behavior).

5.2. Effect of the $\varepsilon$, $a_0$, and $a_2$ on the ratios prescribed to the secretion signal.

Effect of $\varepsilon$. As explained in [5], for $a_1$ small enough, we can approximate the period of the GnRH Secreting System, for any value of $X$ ranging between $-2\mu$ and $-\mu$, by
Figure 17. Constraint 3: The GnRH Secreting System must remain in the surge mode until the Regulating System switches to the fast motion from $P_g^+$ to $Q_g^-$. For $X = \mu$ (left panel), the $\dot{y}$-nullcline must lie in the closed nonhatched half-plane (right panel). Hence, we have to impose that the extremum $P_f^-$ lies on the left of the $\dot{y}$-nullcline, i.e., for $X = -\mu$, $\dot{y} > 0$ at $P_f^-$. 

\begin{align}
(5.5) \quad \varepsilon \left( \int_{-2\lambda}^{-\lambda} \frac{f'(x)}{a_0 x + a_1 f(x) + a_2 + cX} \, dx + \int_{2\lambda}^{\lambda} \frac{f'(x)}{a_0 x + a_1 f(x) + a_2 + cX} \, dx \right).
\end{align}

Under conditions (5.1) and (5.4), this period is both well defined and finite. We can deduce from expression (5.5) that the pulse frequency increases as $\varepsilon$ decreases. Similarly, the surge amplitude increases as $\varepsilon$ decreases, since the $(x,y)$ point moves along the left branch of the cubic $y = f(x)$ at an $O(1/\varepsilon)$-speed, compared to the $O(1)$-speed of the $Y$ motion that we use as reference.

**Effect of $a_0$ and $a_2$.** The surge amplitude increases exponentially as $a_0$ decreases. Thanks to this exponential dependency, it is easy to find an $a_0$ value compatible with condition (5.1) and leading to a high-amplitude surge. $a_2$ controls the position of the stripe described by the $\dot{y}$-nullcline in the $(x,y)$-plane, as $X$ increases from $-2\mu$ to $-\mu$. When $a_2$ increases, the stripe moves leftward in the $(x,y)$-plane, so that both the pulse frequency and the surge amplitude increase.

**Effect of $c$.** $c$ controls the width of this stripe. Since conditions (5.1) and (5.4) drastically reduce the range of admissible values for $c$, they also lower its influence on the $y$ dynamics along the left branch of the cubic $y = f(x)$. Hence, changes in $c$ do not much impact the surge. In contrast, a small change in the width of the stripe strongly impacts the set of limit cycles followed by $(x,y)$ as $X$ increases from $-2\mu$ to $-\mu$. Finally, the greater $c$ is (even subject to condition (5.1)), the greater are the stripe width and the pulse frequency ratio between the beginning and the end of the pulsatility phase. The animation (7322306.avi [184KB]) linked to Figure 18 emphasizes the increase in the pulsatility frequency as the $\dot{y}$-nullcline moves leftward. It illustrates the possibility of tuning the width of this stripe as $X$ increases from $-2\mu$ to $-\mu$ and obtaining a prescribed pulsatility frequency ratio between the beginning and the end of the pulsatility phase.
Figure 18. Animated motion of $(x, y)$ (pink, green, and blue diamonds) along the GnRH Secreting System with $X$ fixed to three different constant values. In the first case, the $\dot{y}$-nullcline (pink straight line) crosses the cubic $y = f(x)$ near its right knee. In the second case, the $\dot{y}$-nullcline (green straight line) crosses the cubic between the origin and the right knee. In the third case, the $\dot{y}$-nullcline (blue straight line) crosses the cubic at the origin. The initial data $(x, y)$ is the same in the three cases. The pink (resp., green, blue) diamond describes the limit cycle with a period around 1100 (resp., 800, 640): This situation corresponds to the low pulsatility frequency of GnRH secretion at the beginning of the pulsatile phase (resp., in the middle of the pulsatile phase, at the end of the pulsatile phase). Clicking on the above image displays the accompanying animation (73223_06.avi [184KB]).

5.3. Procedure for tuning the parameter values. As explained in section 2.4, we have fixed once and for all the values of $\delta$, $\lambda$, and $\mu$. More precisely, $\delta = 0.0125$ and the values of $\lambda$ ($\lambda = \sqrt{30}/6$) and $\mu$ ($\mu = 2\sqrt{3}/3$) correspond to cubic functions $f$ and $g$ expressed as

$$f(x) = -x^3 + 2.5x,$$

$$g(X) = -X^3 + 4X.$$  

(5.6a)

(5.6b)

We propose here an algorithm-like procedure for tuning the other parameters in order to meet the set of quantitative specifications together.

The procedure first consists in obtaining an initial guess for the parameters values, from the following specification-driven and analysis-based operating sequence:

1. Fix the value of $a_2$, since this parameter has multiple influences on the different ratios.

To ensure that conditions (5.1) and (5.4) will be fulfilled, we have to position the $\dot{y}$-nullcline precisely in the $(x, y)$-plane in the uncoupled case ($X = 0$), so that it separates the $P^f$ extremum, on its left, from the intersection point between the $x$-axis and the cubic $y = f(x)$, on its right. The corresponding $a_2$ values range between $\lambda$ and
\( \lambda \sqrt{3} \). Within this interval, \( a_2 \) will be even greater than the prescribed surge amplitude is high.

2. Choose the order of magnitude of \( \varepsilon \) to fit the average pulse frequency. Combining the whole cycle duration with the average pulse frequency, we can get an approximate number of pulses along a cycle. Since \( \varepsilon \) represents the difference in the time speed between the Regulating System (which drives the whole cycle) and the GnRH Secreting System (which produces the pulses), \( \varepsilon \) is on the order of the inverse of the pulse number.

3. Tune the value of \( a_0 \) to obtain a suitable frequency at the end of the pulsatility phase. Assuming that \( a_1 \) is small, we may approximate the minimum period of the GnRH Secreting System, for \( X \) ranging between \(-2\mu\) and \(-\mu\), by

\[
(5.7) \quad T_{\text{min}} = 2\varepsilon \int_{-2\lambda}^{-\lambda} \frac{f'(x)}{a_0x} dx = \frac{\varepsilon}{a_0} (9\lambda^2 - 6 \ln 2).
\]

Hence, to obtain a prescribed frequency \( \phi \) at the end of the pulsatility phase, we can link \( a_0 \) to \( \varepsilon \) by

\[
(5.8) \quad T_{\text{min}} = 1/\phi.
\]

The corresponding value of \( a_0 \) will impact on the pulse to surge amplitude ratio, since, as explained in section 5.2, the surge amplitude increases exponentially as \( a_0 \) decreases.

4. Find the value of \( c \) consistent with the pulse frequency ratio \( \rho \) between the beginning and the end of the pulsatility phase. With \( a_2 \) ranging between \( \lambda \) and \( \lambda \sqrt{3} \), the period of the limit cycle of the GnRH Secreting System for \( X = -\mu \) can be approximated by the minimum \( T_{\text{min}} \), whatever the value of \( c \) subject to conditions (5.1) and (5.4). In that case, the period of the limit cycle for \( X = -2\mu \) is equal to \( T_{\text{min}}/\rho \). Finding \( c \) thus amounts to solving the implicit equation

\[
(5.9) \quad \int_{-2\lambda}^{-\lambda} \frac{f'(x)}{a_0x + a_1 f(x) + a_2 - c\mu} dx + \int_{2\lambda}^{\lambda} \frac{f'(x)}{a_0x + a_1 f(x) + a_2 - c\mu} dx = \frac{T_{\text{min}}}{\rho}.
\]

It is worth noticing that this equation does not admit a solution in \( c \) for every value of \( \rho \). The greatest ratio can be reached with the following value of \( c \):

\[
(5.10) \quad c = \frac{a_0\lambda + 2a_1\lambda^3 + a_2}{2\mu},
\]

which corresponds to the bound of the constraint (5.1).

5. Define the precise value of \( a_1 \). We have already assumed that \( a_1 \) is small enough (in a sense detailed in section 5.1). The precise choice of \( a_1 \) marginally affects the amplitude of the surge, which increases as \( a_1 \) increases.

6. Deduce the values of \( b_1 \) and \( b_2 \) from the results established in section 4. For a prescribed duration ratio \( r \) between the pulsatility phase and the surge durations, there is a one-dimensional curve of solutions in the \((b_1,b_2)\) space. Along one such curve of constant ratio, the smaller \( b_1 \) is (within the \([\hat{b}_1,\frac{1}{r}]\) interval), the longer \( X \) remains close to \(-\mu\), in comparison with the time spent near \( X = -2\mu \), and hence the sooner the pulse
frequency increases. This property is used to approximately set the durations of the luteal and follicular phases, even if it is not straightforward to determine the value of $X$ (between $-2\mu$ and $-\mu$) for which the pulse frequency starts increasing drastically.

From this initial guess, numerical simulations are then performed to gradually improve the compliance with the specifications. More precisely, the values of $\varepsilon$ and $a_0$ are updated from the comparison of the observed $y(t)$ signal with that of the prescribed signal. The value of $\varepsilon$ need only be updated once (its initial value is simply multiplied by the ratio of the prescribed to the observed number of pulses along the cycle). In contrast, since the search for $a_0$ is a little more heuristic, several trial simulations may be needed before obtaining the expected pulse to surge amplitude ratio. Finally, the remaining parameter values are fixed from directly entering Step 4 in the sequence described above.

In section 6.1.3, we bring this procedure into play in great detail.

6. Quantitative neuroendocrinological specifications.

6.1. GnRH secretion along the ovarian cycle in the ovine species.

6.1.1. Duration of ovarian cycle phases. During the breeding season (late summer to the start of the next spring), the ewe shows ovarian (estrous) cycles of 16–17 days [13]. The cycle is divided into 2 phases, the follicular and the luteal phases. The follicular phase has a duration of 2–3 days and is characterized by increasing secretion of estradiol and onset of the LH (luteinizing hormone) surge (triggered by the GnRH surge). The luteal phase has a duration close to 14 days and is characterized by the secretion of progesterone from the corpus luteum. The transition from the follicular phase to the luteal phase is marked by ovulation. In the absence of pregnancy, the transition from the luteal phase to the follicular phase is marked by the luteolysis of the corpus luteum, allowing resumption of a new cycle.

6.1.2. Amplitude and frequency ratios. We first detail the specifications concerning the ovine species, since this is the species for which there are more data available directly on the GnRH level (rather than the LH level). Indeed, in the ewe, the development of a dedicated technique has allowed the sampling of pituitary portal blood with high temporal resolution [16]. This technique is especially useful in studying the pattern of GnRH secretion during the surge. It has been utilized both during the luteal and follicular phases of naturally cycling animals [17] and during artificial, estradiol-induced, follicular phases in ovariectomized ewes [17, 15]. In any case, the LH surge induced by estradiol was invariably accompanied by a massive GnRH surge.

The peak values of the GnRH surge\(^2\) are subject to a huge between-animal and between-study variability [16, 17, 15]. This variability ensues mainly from the complexity of the experimental procedure (for instance, the amplitude is dependent on the anatomical level of the surgical lesion into the portal vessels) and the physiological status of the ewes (either natural or induced ovarian cycles). On the whole, the criteria applied to define the surge and compute its amplitude tend to lower the maximal surge amplitude that we need to fix our ratio. To cope with this variability, we based our study on the maximum experimental

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\(^2\)In the cited papers, the surge peak value is actually an average computed from the values observed during 1 hour after the maximum has occurred. Hence, with a 10 min sampling, this is an average over 6 values.
peak values, which are expressed as a 500-fold increase over baseline, to get a surge amplitude specification. We next needed to fix the pulse amplitude. For lack of anything better, we used a visual examination of the raw data (provided by Figures 4 and 5 in [16]) to state that the average peak values can be assessed as less than 10-fold the baseline. We selected a slightly lower pulse value (close to 8.5-fold the baseline) to end up fixing the pulse to surge ratio of 1 to 60.

The experimental studies had also established that the GnRH pulse frequency not only was greater in the early follicular phase than in the luteal phase but also increased further within the follicular phase as the surge approached. More precisely, during the luteal phase, the average frequency was 1 pulse per 4 hours, while it exceeded 1 pulse per hour in the follicular phase. Accordingly, we considered an average pulse frequency ratio of 1 to 4 between the luteal and follicular phase in the model.

6.1.3. Parameter combination for the ovine cycle. Gathering the information detailed above, we set ourselves the target of meeting the specifications detailed in Table 2.

<table>
<thead>
<tr>
<th>Feature</th>
<th>Specification</th>
<th>Unit</th>
</tr>
</thead>
<tbody>
<tr>
<td>whole cycle duration</td>
<td>16.5</td>
<td>days</td>
</tr>
<tr>
<td>follicular phase duration (surge excepted)</td>
<td>2.5</td>
<td>days</td>
</tr>
<tr>
<td>surge duration</td>
<td>1</td>
<td>days</td>
</tr>
<tr>
<td>luteal phase duration</td>
<td>13</td>
<td>days</td>
</tr>
<tr>
<td>pulse to surge amplitude ratio</td>
<td>1 to 60</td>
<td>–</td>
</tr>
<tr>
<td>frequency increase ratio</td>
<td>1 to 4</td>
<td>–</td>
</tr>
</tbody>
</table>

We now apply the procedure described in section 5.3 to find the corresponding values. *Initial guess for* $a_2$, $\varepsilon$, $a_0$, $c$, $a_1$, $b_1$, and $b_2$ *from the sequence described in section 5.3*. For each step of the sequence, we recall the involved input specification and we compute the resulting parameter output.

1. Input specification: pulse to surge amplitude ratio
   Parameter output: $a_2$
   Since the surge amplitude in the ewe is expected to be medium-sized, we can choose a value of $a_2$ in the middle of the range $[\lambda, \lambda \sqrt{3}] \simeq [0.913, 1.581]$, for instance, $a_2 = 1.14$.

2. Input specification: whole cycle duration and average pulse frequency
   Parameter output: $\varepsilon$
   The pulse frequency is expected to increase from 1 pulse per 4 hours during the luteal phase (13 days long) to a maximum of 1 per hour at the end of the follicular phase (2.5 days long). Considering an approximate number of 100 pulses along 1 cycle, we first set $\varepsilon = 0.01$ (which implies that the oscillations of $(x,y)$ around the limit cycle of the GnRH Secreting System are 100 times faster than the oscillations of $(X,Y)$ around the limit cycle of the Regulating System). We recall that this initial value gives only a rough estimate of the appropriate value of $\varepsilon$.

3. Input specification: pulse frequency at the end of the pulsatility phase
   Parameter output: $a_0$
   The maximum frequency (1 pulse per hour) corresponds to the occurrence of 24 pulses
per day. It can be approximated as the inverse of \( T_{\text{min}} \) defined in (5.7). Hence \( T_{\text{min}} = 1/24 \), and
\[
\frac{\varepsilon}{a_0 (9\lambda^2 - 6 \ln 2)} = \frac{1}{24}
\]
so that \( a_0 \approx 0.45 \).

4. Input specification: pulse frequency ratio (\( \rho \)) from the beginning to the end of the follicular phase

Parameter output: \( c \)

A guess value for \( c \) can first be found from the numerical resolution of (5.9), with \( a_1 = 0 \) and \( \rho = 1/4 \). Yet, such a value is not compatible with constraint (5.1) (we recall here that \( c \) is subject to both constraints (5.1) and (5.4)). Hence we have to use (5.10):
\[
c = \frac{a_0 \lambda + a_2}{2\mu} \approx 0.67.
\]

5. Input specification: surge amplitude

Parameter output: \( a_1 \)

Since the expected pulse to surge ratio is on the order of 1 to 60 and the pulse amplitude is given by \( 2\mu^3 \approx 1.52 \), variable \( y \) should reach a peak value around 100 (i.e., \( x \approx -4.6 \) and \( y \approx f(-4.6) \approx 100 \)). Besides, the point \((-4.6, 100)\) has to be on the left of the \( \dot{y} \)-nullcline to ensure the resumption of the pulsatile regime (i.e., for \( X \) around \(-2\mu \)). Hence, with the ongoing set of parameter values \((a_0 = 0.45, a_2 = 1.14, c = 0.67, \epsilon = 0.01)\), \( a_1 \) has to satisfy
\[
-4.6a_0 + 100a_1 + a_2 - 2\mu c < 0, \text{ i.e., } a_1 \lesssim 0.024.
\]

We take, for instance, \( a_1 = 0.01 \).

6. Input specification: duration ratio (\( r \))

Parameter output: \((b_1, b_2)\)

To define the security plane \( H_p^{-\alpha} \), we take \( \alpha = 0.3 \), which is a sufficiently great value to avoid any canard cycles for all \( \varepsilon < 0.1 \) (see Remark 3.2). As the follicular phase is short compared to the luteal phase, we will take \( b_1 \) close to the lower bound of \([b_1^*, 1/\mu^2]::
\[
b_1 > (\mu + \alpha)/(4\mu^3) \approx 0.24.
\]

Following numerically, along \( \varepsilon = 0.01 \), the leaf \( F_{16.5} \) of the \((\varepsilon, b_1, b_2)\) space foliation defined in Theorem 4.4, we obtain as a solution \((b_1, b_2) = (0.248, 1.518)\).

At this stage of the procedure, we have obtained the initial set of parameter values listed in Table 3. The corresponding \( y(t) \) signal generated from the numerical simulation of the model is characterized by a pulse to surge amplitude ratio of approximately 1 to 165 and a pulse frequency increase from 1 per 30 minutes to 1 per 1.5 hours (see Figure 19).

**Updating of the parameter values.** We now have to modify some of the parameter values to improve the compliance to the prescribed ratios. In particular, we will manage to reduce the pulse frequency increase (which is twice as high as expected) and decrease the surge amplitude in a few steps, combining numerical simulations with Steps 4 to 6 of the sequence described in section 5.3:
Table 3  
Initial set of parameter values corresponding to the specifications of Table 2.

<table>
<thead>
<tr>
<th>$\varepsilon$</th>
<th>$\delta$</th>
<th>$a_0$</th>
<th>$a_1$</th>
<th>$a_2$</th>
<th>$c$</th>
<th>$b_1$</th>
<th>$b_2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.01</td>
<td>0.0125</td>
<td>0.45</td>
<td>0.01</td>
<td>1.14</td>
<td>0.67</td>
<td>0.248</td>
<td>1.518</td>
</tr>
</tbody>
</table>

Figure 19. $y(t)$ signal generated by system (2.2) with the initial guess for the parameter values given in Table 3 (simulation performed with XPP-AUT, http://www.math.pitt.edu/~bard/xpp/xpp.html). The time scale unit is one day. The $y$ variable has no dimension, since we are interested in the pulse to surge amplitude ratio. The blue rectangle represents the zoom range. The signal meets the specifications for the duration of the ovarian cycle in the ewe (16.5 days). The other specifications are not respected yet: The pulse to surge amplitude ratio reaches approximately 1 to 165, while the pulse frequency increases from 1 per 80 minutes to 1 per 30 minutes between the beginning and the end of the pulsatility phase.

Table 4  
Parameter values for the ovarian cycle in the ewe.

<table>
<thead>
<tr>
<th>$\varepsilon$</th>
<th>$\delta$</th>
<th>$a_0$</th>
<th>$a_1$</th>
<th>$a_2$</th>
<th>$c$</th>
<th>$b_1$</th>
<th>$b_2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.02</td>
<td>0.0125</td>
<td>0.52</td>
<td>0.011</td>
<td>1.14</td>
<td>0.70</td>
<td>0.246</td>
<td>1.5103</td>
</tr>
</tbody>
</table>

- The value of $a_2$ is left unchanged, since the order of magnitude of the ratios is appropriate.
- We set $\varepsilon = 0.02$ to divide the pulse frequency by 2. As a consequence, the pulse to surge amplitude ratio gets close to 1 to 90.
- Since this ratio is still too high, we conduct successive numerical simulations with progressively increasing values of $a_0$, until the surge amplitude is reduced to the good ratio, for $a_0 = 0.52$.
- The value of $c$ is computed again with the updated value of $a_0$ (Step 4 of the procedure...
Figure 20. Ovarian cycle in the ewe. \( y(t) \) signal generated by (2.2) with the parameter values given in Table 4 (simulation performed with XPP-AUT). The time scale unit is one day. The \( y \) variable has no dimension as we are interested in the pulse to surge amplitude ratio. The signal respects the specifications for the sheep ovarian cycle: Whole cycle duration of 16.5 days, follicular phase surge excepted (green) of 2.5 days, surge duration (red) of 1 day, and luteal phase (purple) of 13 days. The pulse to surge amplitude ratio is around 1 to 60. The blue rectangle represents the range of Figure 21.

in section 5.3):

\[
c = \frac{a_0 \lambda + a_1 \lambda^3 + a_2}{2\mu} \simeq 0.7.
\]

- To compensate for the slightly too low resulting pulse to surge ratio, \( a_1 \) is marginally increased to 0.011.
- The values of \( b_1 \) and \( b_2 \) are computed again with the updated value of \( \varepsilon \) (Step 6 of the procedure in section 5.3) to obtain \((b_1, b_2) = (0.246, 1.5103)\) and complete the final set of parameter values listed in Table 4.

6.1.4. GnRH secretion pattern in the ewe. The GnRH secretion pattern, obtained with the parameter values listed in Table 4, is illustrated in Figure 20. The zoom around the surge in Figure 21 emphasizes the difference in the pulse frequency at the end of the follicular phase (presurge period) compared to the beginning of the luteal phase (postsurge period).

Figure 21. Zoom around the surge (ewe). \( y(t) \) signal generated by (2.2) with the parameter values given in Table 4. The whole presurge follicular phase (2.5 days) is represented here in green. The pulse frequency before the surge is approximately 1 pulse per 70 minutes. The pulse frequency at the beginning of the luteal phase (purple) is around 1 per 2.5 hours.
6.2. GnRH secretion along the ovarian cycle in the rhesus monkey.

6.2.1. Specifications on cycle phase duration and GnRH surge to pulse ratios. The duration of the ovarian (menstrual) cycle in the rhesus monkey is comparable to that of the human cycle, i.e., around 28 days long. Comparatively to the ovine cycle, it is much more symmetric, since the average duration of both the follicular and the luteal phases amounts to 14 days [4].

GnRH measurements in cerebrospinal fluid (CSF) samples have been obtained from the third ventricle of intact and ovariectomized conscious rhesus monkeys during control periods and throughout an estrogen challenge [21], with a time resolution of 15 minutes. In the intact as well as ovariectomized rhesus monkey, a genuine GnRH surge does occur in response to estradiol, and the profile of the GnRH surge is remarkably similar to that reported in the ewe. This semiquantitative information is quite reliable since simultaneous measurements of GnRH in CSF and portal blood in the ewe have shown that there is good agreement between both techniques at the time of the GnRH surge [18]. However, it is more difficult to extract accurate quantitative information from these data, since the technique of collecting GnRH in the CSF is less reliable than the sampling of pituitary portal blood, due to difficulties in maintaining the required CSF flow uninterruptedly for a long time and in documenting the position of the tip of the collecting cannula. Moreover, the whole surge duration was not entirely covered by the collecting period in several monkeys, and so maximal values may have been missed. Considering the maximal observed GnRH CSF concentration (around 400 pg/ml) and a median pulse amplitude of 16 pg/ml, we can, however, fix the pulse to surge amplitude ratio, for instance, to 1 to 25.

6.2.2. Parameter combination for the rhesus monkey cycle. Gathering the information detailed above, we set ourselves the target of meeting the specifications detailed in Table 6. Applying the procedure described in section 5 in a way similar to what we have done in section 6.1.3, we deduce the set of parameters listed in Table 5. The corresponding GnRH secretion pattern is illustrated in Figure 22. One can notice the difference in the length of the follicular phase compared to that of the ovarian cycle in the ewe. The zoom around the surge in Figure 23 emphasizes the difference in the pulse frequency at the end of the follicular phase (presurge period) compared to the beginning of the luteal phase (postsurge period).

Table 5
Parameter values for the ovarian cycle in the rhesus monkey.

| $\delta$ | 0.0125 |
| $\alpha_0$ | 0.7 |
| $a_1$ | 0.013 |
| $a_2$ | 0.67 |
| $b_1$ | 0.187 |
| $b_2$ | 1.704 |

7. Conclusion and discussion. We have based our study on a concise model reproducing the different GnRH secretory patterns along an ovarian cycle [5]. In this model, the dynamical pattern of GnRH secretion results from the interaction between two neuronal populations: GnRH secreting neurons and regulating neurons, whose paragon can be embodied by Kisspeptin neurons [8]. The latter population integrates much of the ovarian steroid feedback
Table 6
Quantitative specifications for GnRH secretion pattern along the ovarian cycle in the rhesus monkey.

<table>
<thead>
<tr>
<th>Feature</th>
<th>Specification</th>
<th>Unit</th>
</tr>
</thead>
<tbody>
<tr>
<td>whole cycle duration</td>
<td>28</td>
<td>days</td>
</tr>
<tr>
<td>follicular phase duration (surge excepted)</td>
<td>13</td>
<td>days</td>
</tr>
<tr>
<td>surge duration</td>
<td>1</td>
<td>days</td>
</tr>
<tr>
<td>luteal phase duration</td>
<td>14</td>
<td>days</td>
</tr>
<tr>
<td>pulse to surge amplitude ratio</td>
<td>1 to 25</td>
<td>–</td>
</tr>
<tr>
<td>frequency increase ratio</td>
<td>1 to 4</td>
<td>–</td>
</tr>
</tbody>
</table>

Figure 22. Ovarian cycle in the rhesus monkey. $y(t)$ signal generated by (2.2) with the parameter values given in Table 5 (simulation performed with XPP-AUT). The time scale unit is one day. The $y$ variable has no dimension as we are interested in the pulse to surge amplitude ratio. The signal respects the specifications for the rhesus monkey ovarian cycle: Whole cycle duration of 28 days, follicular phase surge excepted (green) of 13 days, surge duration (red) of 1 day, and luteal phase (purple) of 14 days. The pulse to surge amplitude ratio is around 1 to 25. The blue rectangle represents the range of Figure 23.

Figure 23. Zoom around the surge (rhesus monkey). $y(t)$ signal generated by (2.2) with the parameter values given in Table 5. The last three days of the follicular phase (surge excepted) are represented here in green. The pulse frequency before the surge is approximately 1 pulse per 80 minutes. The pulse frequency at the beginning of the luteal phase (purple) is around 1 per 2 hours.

and acts as a slow pacemaker, while the former one is part time excitable and part time fast oscillating. The alternation of pulsatile and surge modes for GnRH secretion, as well as pulse frequency increase as the surge onset gets closer, has been explained in the framework of bifurcation theory.

Few papers have been dedicated to the mathematical modeling of the GnRH pulse generat-
ing system on the hypothalamic level. To our knowledge, none has ever tackled the fascinating question of GnRH surge triggering. This may be due to the complexity of this neuroendocrine system and to the paucity of time series of GnRH secretion. Most authors have thus focused on the downstream level of GnRH-induced LH release from the pituitary gland. A recent, exhaustive review [19] has detailed the different methods used for analyzing pulsatile hormone secretion, including LH pulsatility. Most of them are based on deconvolution principles and aim at recovering an estimated LH secretion rate from time series of the LH plasmatic level. Differences affect the design of the kernel in the deconvolution integral, which may be in either a parametric (e.g., [3]) or a nonparametric (e.g., [6]) form. The most comprehensive approach in the male called on stochastic differential equations (SDEs) to embed the feedback control exerted by testosterone into a stochastic process framework [14]. The first approach to using FitzHugh–Nagumo-type equations fell within the framework of impulse ordinary differential equations (IODEs), based only on the excitable character of these equations without exploiting either their inherent slow-fast dynamics or their ability to generate periodic orbits [2].

In this paper, we have conducted a deeper analysis of our model, with the definite aim of constraining the model outputs (and, more precisely, the output variable corresponding to GnRH secretion), with respect to a physiologically relevant list of specifications. In other words, we have challenged the ability of the model to meet precise quantitative relations on the secretion signal features. Apart from the total duration of the ovarian cycle, which is expressed in physical time, these relations can all be expressed as ratios, regarding (i) surge duration over the whole cycle duration, (ii) the duration of the luteal phase over that of the follicular phase, (iii) pulse amplitude over surge amplitude, and (iv) pulse frequency in the luteal phase compared to the follicular phase. Another instance of construction and analysis of slow-fast models with specified properties was developed within the framework of Mathematical Neurosciences [11]. The authors aimed at proposing a minimal model of a bursting neuron and identifying biophysical parameters that impact burst and spike properties.

We have described in great detail the sequence of bifurcations undergone by the uncoupled FitzHugh–Nagumo subsystems, beyond the usually investigated situation where the slope of the slow variable nullcline is steep. Using singular perturbation theory and dynamical analysis, we have formulated an $\varepsilon$-expansion of the homoclinic bifurcation surface in the $(b_1, b_2, \varepsilon)$ space. From this expansion, we have been able to restrict the space of parameter search precisely, without reducing the set of reachable ratios. Within this restricted space, we have described a foliation, whose leaves define constant duration ratios between the surge and whole cycle. From then on, we have used this foliation to determine a sufficient condition to fulfill the specification on such a ratio. This condition links together the values of 3 parameters $(b_1, b_2, \varepsilon)$ over the 7 to be fixed in the form of the model that we rewrote in the beginning of the article. The remaining parameters can be further tuned according to an algorithm, taking into account the other prescribed ratios. We have managed to cope with all the ratios, even if the frequency increase is structurally limited in the framework of the model.

We have finally applied our results to reproduce the GnRH secretion pattern in two different species in which GnRH data are both directly available and reliable. The ovine species exemplifies an estrous cycle, whose most obvious sign consists of heat behavior, while the rhesus monkey exemplifies a menstrual cycle, whose most obvious sign consists of menstruation. The former species is mostly interesting from an agronomic viewpoint, while the latter
is interesting from a comparative physiology viewpoint, since its ovarian cycle is the closest to the human cycle. Even in species for which fewer GnRH data are available, we can make use of our parameter search algorithm. For instance, if we assume that the GnRH secretion dynamics in cows is comparable to that observed in ewes, as suggested in [10], and adapt the durations of the follicular and luteal phases to values appropriate for the cow [12], we can bend the ovine GnRH signal into a bovine-like signal. In the same spirit, we plan to represent various physiological and pathological situations from our deep understanding of the model behavior.

From a dynamical viewpoint, this study indirectly addresses the questions of tracking homoclinic connections, where classical Canard cycles disappear, and Bogdanov–Takens-like bifurcations of relaxation oscillators that occur on a parameter control manifold. Future work will also focus on determining whether the whole system admits a strictly periodic attractive orbit. It is a challenging question dealing with the synchronization of weakly coupled oscillators and delay to bifurcation analysis.

REFERENCES


