A minimalist model of calcium-voltage coupling in GnRH cells

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Abstract. We present a minimalist model to describe the interplay between burst firing and calcium dynamics in Gonadotropin-releasing hormone (GnRH) cells. This model attempts to give a qualitative representation of Duan's model [3], and it comprises two FitzHugh-Nagumo (FHN) coupled systems describing the dynamics of the membrane potential and calcium concentration in the GnRH cells. Within the framework of our minimalist model, we find that the calcium subsystem drives burst firing by making the voltage subsystem to undergo a Hopf bifurcation. Specifically, fast relaxation oscillations occur in a specific region of the $c-z$ plane ($c$ being the calcium concentration, and $z$ a calcium-dependent gating variable). Slow calcium oscillations, instead, are carried by the voltage subsystem by successive shifts of the calcium steady state, and have the net effect of an external perturbation. The full comprehension of the phase-plane of the voltage subsystem and the 3-dimensional phase-space of the calcium subsystem ultimately allows us to study the behaviours of the entire model under the change of certain parameters. Those special parameters do not necessarily follow realistic assumptions, but merely intend to mimic some pharmacological tests which have been performed experimentally and also simulated by Duan’s model under the corresponding physiological considerations.

1. Introduction

GnRH cells are located in the basal hypothalamus, and are responsible for the production of the GnRH decapeptide. The episodic release of this hormone into the portal system stimulates the pituitary to release gonadotropins, LH and FSH, which drive sexual developments and control the female menstrual cycles and male spermatogenesis. The mechanisms governing GnRH release dynamics are still unknown, and the investigation of those mechanisms could provide some clues to understand central aspects of life such as maturation and fertility.

Based on experimental data recorded by the group of Herbison in GnRH-Pericam transgenic mice [1, 2, 3], Duan et al constructed a detailed model for the behaviour of GnRH cells [3, 4]. Duan’s model relies on the specific molecular components participating in the control of the membrane potential and the intracellular (and endoplasmic reticulum (ER)) calcium concentrations, and the model accurately reproduces the results obtained by Herbison et al. Moreover, the model was tested for different pharmacological conditions, and it was used to predict the existence of a slow activated long-duration potassium current (UCL2077 sensitive $sI_{AHP}$), which was later identified experimentally.
However, the extremely high dimensionality of the system (due to the many gating variables and variables describing the states of the different channels and pumps), makes it cumbersome to undertake a dynamical analysis of the model in a straightforward way\(^1\).

In this work, we attempt to approach Duan’s model starting from an essentially simplified framework. In order to capture only the excitable features of both the membrane potential and calcium concentration dynamics, and the fact that spiking activity in GnRH cells not only drives calcium transients (as is known to be the case) but it is itself driven by the latter, we put aside the detailed underlying biology and represent both burst firing and intracellular calcium concentration via two coupled FHN dynamical systems, and one (calcium-dependent) gating variable controlling voltage.

For such a simplified model, we can set the time scales according to the experimental findings (a fast evolution of the voltage, superimposed on a slow evolution of the calcium concentration). The remaining parameters are set so as to mimic the qualitative features of both the calcium concentration and the membrane potential, as obtained in the experiments or under Duan’s model simulations. Nevertheless, the main goal of this paper is to achieve a full comprehension of the dynamics of the two subsystems, and the interplay between these two dynamics. Such a characterization of the voltage subsystem phase-plane, and the influence of that on the 3-dimensional phase-space associated to the calcium concentration, will eventually allow us to explain the behaviour of the complete model under the change of certain parameters. Those parameter modifications do not necessarily follow realistic assumptions, but are merely an attempt to mimic some pharmacological tests which were performed experimentally to recognize the key molecular components controlling the voltage and the intracellular calcium concentrations in the GnRH cells\(^2\).

2. The minimalist representation of Duan’s model

The full minimalist model for the activity of the GnRH cells is given by two FHN subsystems. The first subsystem concerns the fast voltage dynamics, and it reads,

\[
\begin{align*}
\epsilon_1 \frac{dv}{dt} &= v(\theta_1 - v)(v - \alpha_1) - w_1 + I(c, z) \\
\frac{dw_1}{dt} &= \beta_1(v - \gamma_1 w_1).
\end{align*}
\]

The second subsystem represents the slow dynamics of the calcium concentration, and it involves an extra variable (denoted by ‘\(z\)’) which plays the role of a delay variable,

\[
\begin{align*}
\epsilon_2 \frac{dc}{dt} &= (c - 0.1)(\theta_2 - c)(c - \alpha_2) - w_2 + q(v) \\
\frac{dw_2}{dt} &= \beta_2(c - 0.1 - \gamma_2 w_2) \\
\tau_z(c) \frac{dz}{dt} &= z_\infty(c) - z.
\end{align*}
\]

The fast voltage-subsystem and the slow calcium-subsystem are coupled by means of “current-like” terms, which are as follows,

\(^1\) For a discussion of the bifurcation analysis of Duan’s model, see [4].

\(^2\) The simulations by means of Duan’s model, on the contrary, obey more physiological considerations.
\[ I(c, z) = z \left( 1 - \frac{c^n}{Kd_1^n + c^n} \right) \]

and

\[ q(v) = h_2 \left( \frac{v^{n_2}}{Kd_2^{n_2} + v^{n_2}} \right). \]

The description is complete with the addition of the calcium-dependent functions governing the delay variable evolution,

\[ z_\infty(c) = \exp \left( -\frac{(c - p_2)^2}{b_2} \right) \quad \text{and} \quad \tau_z(c) = f_2 \exp(-d_2 c). \]

The time scales of both subsystems are set by choosing suitable values for the parameters \( \epsilon_1, \epsilon_2, \beta_1, \) and \( \beta_2. \) The full list of the parameters of the model is given in the Table 1.

### Table 1. List of the model parameters.

| index | \( \alpha \) | \( \beta \) | \( \gamma \) | \( \epsilon \) | \( \theta \) | \( n \) | \( Kd \) | \( b \) | \( d \) | \( f \) | \( h \) | \( p \) |
|-------|-------------|-------------|-------------|-------------|-------------|-------|-------|-------|-------|-------|-------|-------|
| 1     | 0.1         | 1.0         | 0.5         | 0.01        | 1.0         | 4     | 0.5   | -     | -     | -     | -     | -     |
| 2     | 0.18        | 5.95 \times 10^{-4} | 1.0         | 0.2         | 0.52        | 4     | 0.5   | 5 \times 10^{-4} | 20.0 | 1.4 \times 10^3 | 0.025 | 0.132 |

Figure 1 displays the simulations (for the standard experimental setup) of Duan’s model and those of the minimalist model.

**Figure 1.** For the regular conditions of Herbison’s experimental setup (left) Duan’s model simulations and (right) the double FHN model simulations.

Even though the shapes of the calcium transients do not match perfectly well, the qualitative features are properly accomplished\(^3\). Namely, intracellular calcium displays slow-scale transients,
whereas the voltage displays fast-scale burst firings composed of 3 or 4 spikes per burst perfectly synchronized with the beginning of the calcium transients. There is only one feature which our model does not account, which is the randomness in the interval between two burst firings. This interburst interval setting is an important issue not only because it is correlated with the amplitude of the next calcium transient (see figure 1), but because is one of the main subjects under the control of the two afterhyperpolarization potassium currents studied in [3]. That failure of our model is a consequence of its fully deterministic character.

However in the double FHN model the relative time scales are controlled so as to reproduce the time scales of the experiments, the absolute value of the time remains measured in arbitrary units. The units of the calcium concentration and the current are arbitrary as well.

3. Dynamical analysis of the double FHN model

The slow subsystem controls the fast subsystem by means of the term $I(c, z)$ (equation (1)). As depicted in figure 2, the effect of this “current” is a shift up and down of the cubic shaped nullcline of the fast subsystem (the $v$-nullcline). Now, for a specific value $I = I_{HB}$ the intersection of the $v$ and $w_1$-nullclines happens exactly at the minimum of the former. Lower values of $I$ mean an intersection to the left of that minimum, whereas higher values mean an intersection to the right. As an intersection to the left corresponds to a stable fixed point and an intersection to the right to an unstable one surrounded by a limit cycle, this special pair of “parameters” $c$ and $z$ (which make the $w_1$-nullcline to intersect the $v$-nullcline at its minimum) represents a Hopf bifurcation of the fast subsystem (see reference [5] for a further explanation). In fact, there is not one pair, but a continuous curve (in the $c-z$ plane) for which $I(c, z) = I_{HB}$, so we have actually a two parameter-dependent Hopf bifurcation separating two specific regions in the parameter-plane (figure 2, right). Region I corresponds to a steady state-regime, and region II to a relaxation oscillations-regime. Most of the time the fast system stays in the region I (close to the steady state), it eventually undergoes the Hopf bifurcation and enters region II (turning around the limit cycle four times), and finally it enters again region I (and approaches asymptotically to the steady state). The bottom panel of figure 2 displays the time series of the voltage during the incursions of the fast subsystem in the region II of the parameter-plane. These short term incursions driven by the slow subsystem are actually the mathematical substrate of the burst firing.

When viewed on the scenario of the slow subsystem, the two parameters ($c$ and $z$) defining the Hopf bifurcation of the fast subsystem have to be regarded as dynamical variables. However, the calcium submodel involves three differential equations (see equation (2)), so now we have to visualize the $c-z$ parameter space of the voltage submodel (figure 2, top-right) as a single piece of a 3-dimensional phase-space, namely, the section $w_2 = constant$. Figure 3 displays the trajectory of the slow subsystem in the full $c-w_2-z$ phase-space. 2-dimensional graphs depict the projections of the trajectory on the planes $c-w_2$ (left) and $c-z$ (right).

Even though the effect of the fast submodel on the slow one can be regarded also as a shift of the cubic-shaped nullcline (represented now by the $c$-nullcline of the calcium 3-dimensional submodel), the way as slow oscillations are generated is different. As depicted in figure 3, the nullclines governing the dynamics in the directions $c$ and $w_2$ keep intersecting to the left of the minimum of the former even when the “current” $q$ saturates near its maximum value $h_2$. As $\epsilon_2$ is small enough for being under the FHN’s model conditions (which means a fast evolution in the $c$ direction and a slow evolution in the $w_2$ direction), the previous remark implies that the fixed point doesn’t change its stability. Yet, when the slow subsystem is perturbed by means of a strong current $q$ (near the top of the voltage spikes), the $c$-nullcline is momentarily

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4 The analysis performed in this section is based on the well known time scale separation techniques and bifurcation analysis. For a detailed overview of the subject, see references [5, 6].
Figure 2. Effect of calcium concentration on the voltage. (Left) For low values of $I(c, z)$ the fast subsystem stays close to a stable fixed point. Beyond the threshold given by $I(c, z) = I_{HB}$, it starts turning around a limit cycle. The corresponding attractors are displayed in orange. (Middle) The Hopf bifurcation curve in the parameter-plane separating two different behaviour regions. Region I corresponds to a steady state regime where the membrane potential is at rest. Region II corresponds to the relaxation oscillations-regime. Short term incursions of the fast subsystem into the region II are the responsible for the spiking activity of the membrane potential (right).

Figure 3. (Left) 3-dimensional phase-space of the slow-subsystem displaying the trajectory of a regular calcium transient (black), the Hopf bifurcation surface (blue) and the $z$-nullcline (green). (Middle and right) Projections of the trajectory on the $c-w_2$ and the $c-z$ planes. The most left and the most right vertical dashed lines enclose the bursting region. In between, a third vertical dashed line denotes the point where the trajectory intersects the resting $c$-nullcline.

'replaced' by a positive cubic-shaped $\dot{c}$ curve, which gives rise to an hypothetical trajectory with the typical shape of that of a perturbed excitable system (namely, an excitable system which has been perturbed enough and consequently performs a large excursion in the phase-space). This mechanism is illustrated in figure 4, where we superimpose to the phase-space (the $c-w_2$ plane only) the contours of the $c$ component of the velocity field. We stress that this trajectory is hypothetical in the sense that the slow system doesn’t complete it as in the case of a usual excitable system, but it follows it only for the very short time for which the current $q$ is large enough, namely, the duration of a single voltage spike. Between spikes, the current $q$ vanishes, the $c$-nullcline returns to its original position, and the $c-w_2$ phase-plane looks again as at the beginning of the burst firing (figure 4, left). Moreover, in these interspike intervals $\dot{c}$ becomes negligible and $z$ starts to be the only relevant direction of movement (figure 3, bottom-right).
until a new spike takes place and the process repeats again.\footnote{As a matter of fact, between spikes new hypothetical trajectories emerge with almost negligible (although negative) initial values of $\dot{c}$. This effect is more evident during the intermediate interspike intervals, and it is a consequence of the fact that, during these intervals the trajectory is slightly above the middle branch of the $c$-nullcline (figure 3, bottom-left panel). Coming again to the comparison with an usual excitable system, this situation would be equivalent to a perturbation below the stability threshold (which in this case is in addition of a short term), and is therefore not relevant for the outcome of the present analysis. Yet, the negative component of the velocity in the $c$ direction is still noticeable in the $c-z$ projection of the trajectory (figure 3, bottom-right panel.)}

**Figure 4.** Superimposed to the $c-w_2$ plane-space of the slow subsystem, the contours of the $c$ component of the velocity field (light blue curves). (Left) Interburst intervals and interspike intervals within a burst firing: $v \simeq 0$, so that $q \simeq 0$ and therefore the $c$-nullcline is at rest. (Right) Near the top of the first spike: $v \gg Kd_2$, so that $q \simeq h_2$ and therefore the $c$-nullcline is replaced by a positive $\dot{c}$ cubic-shaped curve. The dashed curve represents the trajectory followed by the slow subsystem when the voltage is at the top of the spike. In both panels, the purple disks represent the $c-w_2$ projection of the corresponding steady state.

The mechanism described in the previous paragraph repeats several times (as many times as spikes are in one burst), and it is the responsible for the ‘jumps’ of the trajectory in the $c$-direction during the burst firing (see figure 3, bottom-right. In this picture the most left and the most right vertical dashed lines depict the values of $c$ which enclose the burst region, which corresponds to the portion of the trajectory above the Hopf bifurcation surface of the fast system). However, we stress that this mechanism, being necessary, is not enough by itself to generate the experimental large-amplitude calcium transients that the model is capable to simulate. A second mechanism, which has again the feature of the perturbation of an excitable system (but that works in a different manner) is required. In both the left and right panels of figure 3 we plot three vertical dashed lines, the most left and the most right being the specific values of $c$ for which the trajectory intersects the Hopf bifurcation of the fast system. The middle vertical dashed line, on the other hand, depicts the value of $c$ for which the trajectory intersects the $c$-nullcline. This specific value of $c$, which not necessarily coincides with the end of the burst firing region, corresponds to the real slow system’s stability threshold. Namely, once the $c$ variable goes beyond this value, $\dot{c}$ becomes positive regardless the value of the current $q$. Later on, a short term increase of the velocity has a negligible effect on the future trajectory, and the large excursion in the slow phase-space happens regardless what the fast system is doing. The fast system might actually keep spiking, but eventually it will cross again the Hopf bifurcation surface and leave the burst firing region (region II in the central panel of figure 2).

To sum up, fast oscillations are driven by the slow subsystem by means of a calcium-dependent current which set the fast system into the relaxation oscillations-regime. Slow oscillations of large amplitude, instead, are a consequence of the interplay of two similar, but qualitatively different
mechanisms. Both mechanisms are comparable to that of an usual FHN system which, being out of the relaxation oscillations-regime, is perturbed in a certain way. One of these mechanisms is voltage-dependent and it involves a short term increase of the \( c \) component of the velocity. The second mechanism is voltage-independent and means a simple increase of the variable \( c \) (not its time derivative) beyond the middle branch of the FHN’s cubic shaped nullcline, which is actually the result of several realizations of the first mechanism.

4. The behaviour of the minimalist model beyond the regular conditions
In this section we will give three examples which illustrate how the mechanisms controlling the burst firing and the calcium oscillations might fail, and what happens to both subsystems in each situation. Those examples, being the outcome of the modification of some specific parameters, are connected with different pharmacological perturbations of the standard experimental setup. For a detailed discussion of those pharmacological perturbations and the corresponding Duan’s model realizations see [3, 4].

4.1. The Tetrodotoxin test
Tetrodoxin (TTX) is a well known sodium current blocker, and it has therefore the effect of abolishing action potentials in excitable cells. In [3] TTX has been supplied to test whether burst activity initiated the high amplitude calcium transients. As the outcome of the experiment was a total suppression of both burst firing and calcium oscillations, the conclusion was that it actually did.

To prevent the fast subsystem of the minimalist model to oscillate, we only need to avoid the incursions of the fast subsystem trajectory into the relaxation oscillations region of the \( c-z \) parameter-plane (region II in figure 2, bottom right panel). An easy way to accomplish this task, is by increasing the numerical value of \( I_{HB} \), which has the effect of displacing the Hopf bifurcation upwards in the parameter-plane. A direct examination of the left panel of figure 2 immediately suggests that this can be done by simple making the parameter \( \gamma_1 \) equal to zero, as this parameter inversely controls the slope of the \( w_1 \)-nullcline. As a result, the \( w_1 \)-nullcline acquires a vertical slope, and the minimum of the \( c \)-nullcline can not intersect it regardless the value of the actual current \( I(c,z) \). Hence, the region of relaxation oscillations disappears from the parameter-plane. Figure 5 displays the behaviour of the model for such conditions. The limitation of the fast subsystem to reach the relaxation oscillations-regime prevents the slow subsystem to be perturbed by means of the mechanisms described in the previous section, and hence it moves to its steady state causing calcium oscillations to be completely suppressed.

4.2. The Cyclopiazonic Acid test
The roles of calcium internal stores were evaluated by Herbison et al by treating GnRH neurons with 2-aminoethoxydiphenylborate (2-APB) and cyclopiazonic acid (CPA); antagonists at IP\(_3\)R and the sarcoplasmic reticulum calcium-ATPase pump, respectively. They found that 2-APB blocked both calcium transients and burst firings, whereas CPA had the effect of suppressing calcium transient amplitude and making burst firing disorganized[3].

To suppress calcium transients in our minimalist model (allowing voltage activity), we have to focus on the first mechanism controlling the slow subsystem dynamics. By eliminating that mechanism (which in turn causes the suppression of the second mechanism, however this is an indirect effect -we will see shortly the outcome of a direct suppression of the second mechanism), we prevent at all calcium oscillations keeping unaffected the chance of the fast subsystem to develop action potentials. A possible parameter to be perturbed to accomplish such a behaviour is the parameter which controls the amplitude of the current \( q(v) \), namely, \( h_2 \). Figure 6 displays the model simulations for the normal conditions \( (h_2 = 0.025) \), followed by an interval of ‘low extracellular calcium’ \( (h_2 = 0.005) \). During the top of the spike, the current \( q(v) \) makes calcium
to increase even for small value of $q(v)$ (see figure 6, right panel). However, as the current is very week, the positive velocity which calcium acquires during the spikes is comparable to the negative velocity it acquires during the interspike intervals. As a result, calcium performs very tiny, constant average, tent-like oscillations around the baseline, and the trajectory looks as it reaches a steady state in the 3-dimensional phase-space (figure 6). Moreover, as in our simulations the parameter $h_2$ changes abruptly, during a short time-window just after the change $z$ turns out to be the main direction of motion (this is because $\dot{c}$ is small both when it is negative and when it is positive), and therefore the system oscillates around the $z$-nullcline until the actual intersection of the three nullclines is enough approached.

4.3. The Free Calcium test
To analyse the role of external calcium in burst firing generation, Herbison et al replaced calcium in the normal artificial cerebral spinal fluid with equimolar magnesium. As a result they observed a gradual suppression of calcium transients correlated with a failure of the normal burst firing patterns. The reduction of calcium transients correlated with a decrease of interburst intervals, an increase in burst duration and an increase in spikes per burst[3].

Most of free calcium experiment findings are accomplished by the minimalist model when the second mechanism controlling the slow subsystem dynamics is directly suppressed. We want now the first mechanism to work properly, so we will keep the parameter $h_2$ somehow close to the standard conditions (however we will decrease it slightly to enlarge the spikes per burst). As discussed in section 3, the second mechanism means a perturbation of the slow subsystem beyond the threshold given by the middle branch of the $c$-nullcline; it is a voltage independent mechanism (namely, it makes the $c$ component of the velocity positive even if the burst firing have finished), and is the responsible for the high amplitude of the calcium transient. A direct way for suppressing this mechanism is by displacing the middle branch of the $c$-nullcline to the right. This can be done easily by increasing the parameter $\alpha_2$, which defines the position of the middle root of the $c$-nullcline. The results of the simulations are depicted in figure 6.

We stress that in this scenario calcium oscillations actually occur, however the slow system

![Figure 5. Simulations of the TTX test.](image-url)
Figure 6. (Left) Simulations of the CPA test (in the region $h_2 = 0.005$, a small time-window is squeezed to emphasize the behaviour of the model). (Right) Phase-space of the slow subsystem (one regular calcium transient is included before the CPA simulation).

doesn’t reach the threshold represented by the middle branch of the $c$-nullcline neither. This fact is depicted in 7, where we display the trajectory of the slow subsystem in the 3-dimensional phase-space and the corresponding projections in the $c-w_2$ and the $c-z$ planes. Unlike the case of the regular bursting, the trajectory does not intersect now the resting cubic shaped nullcline, and therefore the oscillation of the slow subsystem does not mean a big excursion in the phase-space (compare with figure 3).
Figure 7. (Left) Phase-space of the slow subsystem for the free Calcium test (compare with figure 3). (Middle and right) Projections of the trajectory on the $c-u_2$ and the $c-z$ planes. As in figure 3, the most left and the most right vertical dashed lines enclose the bursting region. The trajectory does not intersect here the resting $c$-nullcline, and thus the middle vertical dashed line is not present.

5. Discussion

We have constructed a minimalist model to reproduce the qualitative features of voltage-calcium coupling in GnRH cells, and have analysed its dynamical behaviour. As a result, we observed that calcium drives burst firing by setting the voltage subsystem to a relaxation oscillation-regime. In turn, the fast voltage oscillations are responsible for the initiation of the calcium transients, however the high amplitude of the transients is a consequence of the capability of the slow subsystem to cross a threshold which is not voltage dependent.

As we stated previously, the parameters of our model are not related to the physiological properties of the GnRH cell. This fact makes it hard to take advantage of the results to extract realistic conclusions. However, as a next step we are focused in linking both Duan’s and the double FHN model further. This will be accomplished by comparing the dynamical properties of the two models. For instance, we are concerned to establish the normal form of the bifurcation of Duan’s fast submodel when burst firing is developed. In our model, this process is associated to a Hopf bifurcation, so we expect to observe this type of bifurcation for the fast piece of Duan’s model. If this assertion turns out to be true, a suitable fitting between both models would reveal the physiological value of the parameters. An approach to the bifurcation structure of Duan’s model is accomplished in [4].

Ideally, this procedure would provide a better understanding of the interplay between the calcium and the membrane voltage activity at a molecular level, in the sense that it would tell us which machineries of the cell are actually crucial to develop the observed behaviours.

Acknowledgments

This work is supported by the Marsden Fund of the Royal Society and the Conicet.

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