Mechanisms for coexistence of two limit cycles in a biochemical model

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Abstract: Recently, a differential model of the p53-Mdm2 biochemical network which shows birhythmicity has been proposed to reproduce the two experimentally observed frequencies of oscillations of p53. We analyze the mechanisms at the origin of this birhythmic behaviour. Our approach is to approximate this continuous non-linear model into a lower dimensional piecewise affine model. We build the first return map to show the occurrence of two stable coexistent limit cycles.

Keywords: Living systems, Piecewise linear analysis, Oscillation, Bistability, Model reduction.

1. INTRODUCTION

One mode of complex oscillatory behavior is the coexistence between two stable limit cycles for the same external conditions. This phenomenon, called birhythmicity (Goldbeter, 1996; Decroly and Goldbeter, 1982), is the counterpart of bistability for oscillatory behaviors. Such a behavior has been observed in a number of chemical oscillatory systems (Alamgir and Epstein, 1983; Citri and Epstein, 1988) but, although some studies suggest its occurrence in the heart and the neuronal system (Hounsgaard et al., 1988), birhythmicity has not yet been firmly observed experimentally in biological systems.

Recent experiments performed in the p53-Mdm2 network, one of the key protein module involved in the control of proliferation of abnormal cells in mammals (Ventura et al.;, 2007; Vogelstein et al., 2000, Vousden and Lane, 2007), reported two oscillatory regimes of p53 and Mdm2 in irradiated cells (Geva-Zatorsky et al., 2006) a low-frequency oscillatory regime at low irradiation dose with a period of about 10h and high-frequency oscillations at high irradiation dose with a period of about 6h. This observation raised the question of the existence of birhythmicity in the p53-Mdm2 network which would be at the origin of the two oscillatory regimes experimentally observed as a function of the irradiation dose.

A theoretical answer to this question has been recently brought by Ouattara, Abou-Jaoudé and Kaufman (Abou-Jaoudé et al., 2009; Ouattara et al., 2010). In the framework of a 3-dimensional differential model of the p53-Mdm2 network, they showed that this system could display birhythmicity for a certain range of irradiation dose (Abou-Jaoudé et al., 2009). Thanks to the presence of this birhythmic behavior, they could reproduce the two experimentally frequencies observed as well as the increase of the fraction of cells oscillating with a high frequency when the irradiation dose increases and the changes in the oscillation frequency in the course of the response that have been observed for some cells after irradiation (Abou-Jaoudé et al., 2009; Ouattara et al., 2010).

Here we investigate the mechanisms at the origin of birhythmicity in Ouattara, Abou-Jaoudé and Kaufman’s model (OAK model). As this 3-dimensional continuous non-linear model is difficult to analyze, we approximated it, in a first step, into a 3-dimensional piecewise affine model where the Hill functions have been replaced by step functions and, in a second step, into a 2-dimensional piecewise affine model by setting one autonomous variable as a constant in each domain of the phase space delimited by the thresholds of the step functions. From the 2-dimensional model, we build the first return map and show the coexistence of two stable limit cycles by analyzing the existence and the stability of the fixed points of the return map. A mechanism based on the phase space structure of the system is proposed to explain the emergence of birhythmicity.

2. BIRHYTHMICITY IN THE OAK MODEL

We start from a reduced version of the OAK Model (see equations of the OAK model in Ouattara et al. (2010)):

\[
\begin{align*}
\frac{dP}{dt} &= k_P \frac{K_P}{K_P + M_n} - d_P P \\
\frac{dM_C}{dt} &= k_{M_C} + k_{M_C}^* \frac{P^*}{K_{M_C} + P^*} \left( k_{M_C} - k_{M_C}^* \frac{P^*}{K_{M_C} + P^*} \right) Mc - d_{M_C} M_C \\
\frac{dM_n}{dt} &= V_c \left( k_{M_n} - k_{M_n}^* \frac{P^*}{K_{M_n} + P^*} \right) Mc - d_{M_n} M_n
\end{align*}
\]

(Model 1)
where $P$, $M_c$ and $M_n$ represent the concentration of p53, cytoplasmic Mdm2 and nuclear Mdm2 respectively. $k_P$, $k_{M_c}$ and $k_{M_n}$ (resp. $d_P$, $d_{M_c}$ and $d_{M_n}$) represent the protein production rates (resp. protein degradation rates). $k_a$ and $k_{a,in}$ refer to the parameters characterizing the translocation of Mdm2. $K_P$, $K_{M_c}$ and $K_{M_n}$ represent the threshold constants. Finally, $V$ is the cytoplasmic to nuclear volume ratio.

For appropriate parameter settings, Model 1 presents the same bifurcation picture as a function of nuclear Mdm2 degradation rate ($d_{M_n}$) as the OAK Model (see Fig.2b in Ouattara et al. (2010)). In particular, in the oscillatory domain, Model 1 shows two limit cycles, a large amplitude limit cycle of low frequency for low $d_{M_n}$ values (low irradiation dose) and a small amplitude limit cycle of high frequency for high $d_{M_n}$ values (high irradiation dose), separated by a birhythmic region where these two limit cycles coexist. Interestingly, the orbit of the small amplitude limit cycle has a regular shape whereas the projection of the orbit of the large amplitude limit cycle in the plane $(M_n,P)$ contains a long "tail" (Fig.1).

![Fig.1. Projection of the two coexistent limit cycles (solid lines) on the plane $(M_n,P)$ for Model 1. The thresholds $K_{M_n}$ and $K_{M_n}$ related to $P$ are indicated in dashed lines. The parameter values are the same as for the OAK Model (see Table 1 in Ouattara et al., 2010) ($K_{M_n}=0.1$ nM) except: $n=6$, $d_P=2.5$ h$^{-1}$, $K_{M_n}=0.4$ nM and $d_{M_n}=1.9$ h$^{-1}$.

3. FROM CONTINUOUS NONLINEAR TO PIECEWISE AFFINE MODELS

General analytical results concerning the existence and stability of limit cycles for Model 1 can be derived by noting that Model 1 is equivalent to a competitive system. Theorems can be applied to show the existence of a stable periodic orbit (Hirsch, 1988; Zhu and Smith, 1994) for $d_{M_n}$ values for which the system admits a single unstable equilibrium point. However, it is difficult to obtain more information about the oscillatory dynamics of the system. For example, proving the existence of birhythmicity is, to our knowledge, not possible.

Analyzing the mechanisms leading to the emergence of birhythmicity in the framework of this continuous nonlinear system is very challenging. However, (1) involves several highly non-linear terms of the Hill form:

$$\psi(x) = \frac{v^n}{v^n + x^n}$$

for increasing Hill functions.

We thus approximated (1) by replacing these terms by step functions with two levels:

$$s^-(x,v) = 0 \text{ if } X \leq v$$

$$s^+(x,v) = 1 \text{ if } X > v$$

for increasing Hill functions and:

$$s^-(x,v) = 1 - s^+(x,v)$$

for decreasing Hill functions, where $v$ represents now the threshold of the step functions.

This approximation leads to a piecewise affine differential model:

$$\frac{dP}{dt} = k_P s^-(M_n,K_P) - d_P P$$

$$\frac{dM_c}{dt} = k_{M_c} + k_{M_c} s^+(P,K_{M_c}) - (k_a - k_a s^+(P,K_{M_n})) M_c - d_{M_c} M_c$$

$$\frac{dM_n}{dt} = V \left( k_a - k_a s^+(P,K_{M_n}) \right) M_c - d_{M_n} M_n$$

(Model 2)

The space of variables can be decomposed into 6 domains $(D^1, D^2, D^3, D^4, D^5, D^6)$ delimited by the threshold values of the step functions: $K_P$, $K_{M_c}$ and $K_{M_n}$ (Fig.2). In each domain, the equations are affine and stable and one can calculate a so-called target equilibrium point of the domain towards which the system will tend. Importantly, the equation of $M_n$ depends on $M_c$. It follows that the sign of the derivative of $M_n$ can change in each domain of the phase space according to $M_c$.

For appropriate parameter settings, numerical simulations show that the bifurcation diagram as a function of $d_{M_n}$ is similar to the bifurcation picture of Model 1 (not shown). In particular, Model 2 shows birhythmicity with the coexistence of two limit cycles (Fig.2).

$^2$The target equilibrium point is an analogous of the focal point defined in a class of piecewise linear diagonal models (Glass and Pasternack, 1978) (see next section).

$^3$The transition graph, which is the graph defining the possible transitions between the different domains (Sousa, 1989), can thus not be directly derived from the position of the target equilibrium points (see next section).
Fig. 2. Projection of the two limit cycles composing the birhythmic behavior (solid lines) of Model 2 in the plane (Mn,P). The arrows are the transitions between the phase space domains D^9 followed by the two limit cycles. The dashed lines represent the thresholds of the step functions: K_{Mn} and K_{Mc} for P, K_{P} for Mn. The parameter values are the same as for Fig.1 (K_{Mn}=0.1 nM, K_{P}=0.2 nM) except K_{Mc} which has been set to 0.6 nM to reproduce birhythmicity.

- a small amplitude limit cycle of short period, crossing domains D^{12}, D^{22}, D^{13} and D^{23}, in which K_{Mc} threshold is not functional (i.e. P>K_{Mc} along the orbit). This periodic orbit corresponds to the small amplitude oscillatory regime appearing in Model 1 (Fig.1);
- a large amplitude limit cycle of long period passing through all the domains of the phase space. This periodic orbit corresponds to the large amplitude oscillatory regime of Model 1 (Fig.1) and contains a “tail” located in domain D^{21}. As also observed for Model 1, this “tail” contains two folds where the sign of dMn/dt changes: a fold in the transition between D^{22} and D^{21} and a fold inside domain D^{23} (Fig.2).

From this analysis of the orbits of the two limit cycles, two qualitative features characterizing the birhythmic behavior can thus be extracted:
- the presence of two embedded cycles in the graph showing the transitions between the phase space domains followed by the two oscillatory regimes (Fig.2, arrows);
- the presence of a “tail” containing two folds in the orbit of the high amplitude limit cycle.

4. ANALYSIS OF THE BASIC MECHANISMS FOR THE EMERGENCE OF BIRHYTHMICITY

4.1 A two-dimensional reduced model

In order to get more insight into the basic mechanisms leading to birhythmicity, we looked for a further approximation of the previous 3-dimensional Model 2 which would preserve the birhythmic behavior as well as the characteristics of the orbits of the two limit cycles underlined by the previous analysis. In Model 2, in each domain, the evolution of Mn depends on Mc whereas the evolution of Mc does not depend on the other variables of the system (see (2)). We thus considered Mc as a forcing external parameter applied on the evolution of Mn. In this respect, we set Mc as a constant Mc in each domain D^9 of the phase space under some constraints on the Mc values which will be detailed further.

This approximation leads to a 2-dimensional piecewise affine differential model describing our system in which, in each domain of the phase space delimited by the thresholds K_{Mc}, K_{Mn} and K_{P}, the evolution of each variable is of the form:

\[ \frac{dx}{dt} = k_x - d \cdot x \]

where x represents the level of P or Mn, k_x is a constant which depends on the domain D^9 of the phase space and d is the degradation rate of P or Mn. This type of piecewise diagonal affine systems belongs to a class of dynamical systems proposed originally by Glass and Kauffman (Glass and Kauffman, 1973). Interestingly, these systems have mathematical properties which favor the qualitative analysis of their dynamics (Snoussi, 1989; Glass, 1975; Edwards, 2000; Casey et al., 2006). In particular, one can derive the so-called transition graph, which describes all the possible transitions between the different domains, from the position of the target equilibrium points of each domain (called the focal points) (de Jong et al., 2004; Snoussi, 1989; Glass, 1975; Casey et al., 2006).

In order to reproduce the two folds forming the “tail” which characterize the large amplitude limit cycle in Model 1 and Model 2, we added another threshold K (K<K_{Mc}) for p53 level. As the evolution of Mn in each domain is now monotone, the introduction of this new threshold allows changing the sign of the derivative of Mn as the system crosses threshold K and thus recovering in particular the fold observed in domain D^{21} in Model 2 (Fig.2). The space of variables can thus be decomposed into 8 domains (D^{11}, D^{12}, D^{13}, D^{14}, D^{21}, D^{22}, D^{23}, D^{24}), defined by the thresholds K_{Mc}, K_{Mn} plus the additional threshold K for p53, and K_{P} for nuclear Md2 (Fig.3), in which the equations of evolution are:

\[ \frac{dP}{dt} = -d_P \cdot P \quad \text{in domains D}^i \text{ for } i=2 \text{ and } j=1,2,3,4 \]

\[ \frac{dP}{dt} = k_P - d_P \cdot P \quad \text{in domains D}^i \text{ for } i=1 \text{ and } j=1,2,3,4 \]  (3)

\[ \frac{dMn}{dt} = V_{r} \cdot k_{m'} \cdot M_{cj} - d_{Mc} \cdot Mn \quad \text{in domains D}^i \text{ for } i=1,2 \text{ and } j=1,2 \]

\[ \frac{dMn}{dt} = V_{r} \cdot (k_{m'} - k_{m}) \cdot M_{cj} - d_{Mc} \cdot Mn \quad \text{in domains D}^i \text{ for } i=1,2 \text{ and } j=3,4 \]

(Model 3)

Moreover, in order to keep the basic characteristics of the birhythmic behavior shown in Model 2, we imposed the following constraints on the parameters values of Model 3:
- constraint (1): the transition graph of Model 3 contains the transitions between the different domains followed by the two limit cycles in Model 2 (Fig.3, arrows). This constraint on the transition graph sets constraints on the focal points and thus on the parameter values of the Model 3 (Table 1);
- constraint (2): the setting of the parameters Mcj in each domain D^9 of the phase space of Model 3 has to be in accordance with the evolution of Mc in the two limit cycles from one domain to another in Model 2.

Finally, in order to simplify the calculation of the return map (see section 4.3), the values of the degradation rate, d_{Mn} and d_P, have been chosen to be the same. In this case, the trajectories in each domain of the phase space are straight lines, which greatly reduces the computation of the return map. However, considering different degradation rate values does not change the main results of our analysis (not shown).
4.2 Numerical simulations of the 2-dimensional model

For appropriate parameter settings respecting the constraints stated before, numerical simulations show that the system presents a birhythmic behavior whose phase portrait is similar to the projection in the plane (Mn,P) of the phase portrait of the birhythmicity observed in Model 2 (Fig.2).

Table 1. Conditions on the focal points to obtain the transition graph indicated in Fig.3.

<table>
<thead>
<tr>
<th>Domain</th>
<th>Focal point ((p^i_{ij}, m^i_{ij}))</th>
<th>Conditions on the focal point</th>
</tr>
</thead>
<tbody>
<tr>
<td>D^11</td>
<td>(p^{11} = \frac{k_p}{d_p}, m^{11} = \frac{V \cdot k_p \cdot Mc_{11}}{d_{st}})</td>
<td>(p^{11} \geq K, m^{11} \leq K_p)</td>
</tr>
<tr>
<td>D^21</td>
<td>(p^{21} = 0, m^{21} = \frac{V \cdot k_p \cdot Mc_{21}}{d_{st}})</td>
<td>(p^{21} \leq K, m^{21} \leq K_p)</td>
</tr>
<tr>
<td>D^31</td>
<td>(p^{31} = \frac{k_p}{d_p}, m^{31} = \frac{V \cdot k_p \cdot Mc_{31}}{d_{st}})</td>
<td>(p^{31} \geq K_M, m^{31} \geq K_p)</td>
</tr>
<tr>
<td>D^22</td>
<td>(p^{22} = 0, m^{22} = \frac{V \cdot k_p \cdot Mc_{22}}{d_{st}})</td>
<td>(p^{22} \leq K, m^{22} \leq K_p)</td>
</tr>
<tr>
<td>D^32</td>
<td>(p^{32} = \frac{k_p}{d_p}, m^{32} = \frac{V \cdot k_p \cdot Mc_{32}}{d_{st}})</td>
<td>(p^{32} \geq K_M, m^{32} \geq K_p)</td>
</tr>
<tr>
<td>D^33</td>
<td>(p^{33} = \frac{k_p}{d_p}, m^{33} = \frac{V \cdot k_p \cdot Mc_{33}}{d_{st}})</td>
<td>(p^{33} \geq K_M, m^{33} \geq K_p)</td>
</tr>
<tr>
<td>D^23</td>
<td>(p^{23} = 0, m^{23} = \frac{V \cdot k_p \cdot Mc_{23}}{d_{st}})</td>
<td>(p^{23} \leq K_M, m^{23} \leq K_p)</td>
</tr>
<tr>
<td>D^34</td>
<td>(p^{34} = \frac{k_p}{d_p}, m^{34} = \frac{V \cdot k_p \cdot Mc_{34}}{d_{st}})</td>
<td>(p^{34} \geq K_M, m^{34} \geq K_p)</td>
</tr>
<tr>
<td>D^24</td>
<td>(p^{24} = 0, m^{24} = \frac{V \cdot k_p \cdot Mc_{24}}{d_{st}})</td>
<td>(p^{24} \leq K_M, m^{24} \leq K_p)</td>
</tr>
</tbody>
</table>

In accordance with constraint (1), the transition graph contains two cycles (Fig.3, arrows), a small cycle, \(D^{14} \rightarrow D^{24} \rightarrow D^{23} \rightarrow D^{13} \rightarrow D^{14}\), embedded in a large one, \(D^{14} \rightarrow D^{24} \rightarrow D^{23} \rightarrow D^{13} \rightarrow D^{12} \rightarrow D^{11} \rightarrow D^{12} \rightarrow D^{13} \rightarrow D^{14}\), with a branching point located in domain \(D^{23}\). The small (resp. large) amplitude limit cycle crosses the domains composing the small (resp. large) cycle. The branching point induces a separatrix in \(D^{23}\), passing through the threshold intersection \((Mn=K_P, P=K_{Ma})\) and the focal point of \(D^{23}\), which delimits the regions in \(D^{23}\) from which the system will either reach domain \(D^{22}\) or domain \(D^{13}\) (Fig.3, dotted line). Finally, the large amplitude cycle contains a “tail” with two folds which appear at two domain transitions: \(D^{23} \rightarrow D^{22}\) when the level of \(P\) crosses \(K_{Ma}\) and \(D^{22} \rightarrow D^{31}\) when the level of \(P\) level crosses the additional threshold \(K\). These folds reproduce the two folds observed in the “tail” of the large amplitude limit cycle in Model 2 (see Fig.2).

Fig.3. Simulation of the two coexistent limit cycles (solid lines) in the phase space for Model 3. The separatrix is indicated in dotted line. The arrows are the transitions composing the transition graph of the model, with the domains \(D^i\) representing the nodes of the graph. The dashed lines represent the thresholds \(K_M, K_{Ma}\) and \(K\) for \(P, K_P\) for \(Mn\). The parameter values are the same as for Fig.2 (\(K_{Ma}=0.1\) nM) except \(K_M=0.4\) nM , \(K_P=2\) nM, \(K=0.05\) nM, \(d_p=3\) h\(^{-1}\), \(Mc_{11}=Mc_{21}=Mc_{12}=0\), \(Mc_{22}=5\) nM, \(Mc_{13}=9\) nM, \(Mc_{23}=11.3\) nM, \(Mc_{14}=Mc_{24}=25\) nM. Note that, since the degradation constants \(d_{Ma}\) and \(d_P\) have the same values, the trajectories in each domain are straight lines.

4.3 Analysis of a first return map of the 2-dimensional model

We now prove the existence of birhythmicity in Model 3. To do so, we analyze the existence and the stability of the fixed points of the first return map (Strogatz, 1994) \(F\) from and to the boundary between the domains \(D^{14}\) and \(D^{24}\) (vertical half-line labeled “Z” in Fig.3) crossed by both limit cycles. We also show that \(F\) has a point of discontinuity.

To compute \(F\), we consider the point \((K_P, K_{Ma}+x_0)\) with \(x_0>0\) as an initial condition and compute the function \(F(x_0)\) such that \((K_P, K_{Ma}+F(x_0))\) is the image of \((K_P, K_{Ma}+x_0)\) on the \(z\) axis after one cycle in the phase space. Since the degradation constants \(d_{Ma}\) and \(d_P\) have the same values, the trajectories in each domain are straight lines containing the focal point of the domain. If the trajectory enters domain \(D^j\) from a point \((x_0,y_0)\) and if \((a^j,b^j)\) is the focal point of \(D^j\), then the trajectory \((x,y)\) in \(D^j\) satisfies:

\[
y = \left(\frac{y_0 - b^j}{x_0 - a^j}\right) \cdot x + \left(\frac{b^j x_0 - a^j y_0}{x_0 - a^j}\right)
\]

As the transition graph contains two embedded cycles which both cross the \(z\) axis (Fig.3), we have to compute \(F\) for each of the two cycles. To derive the conditions on \(x_0\) to follow either the small amplitude or the large amplitude cycle, we first have to compute the image of a point of the \(z\) axis, \((K_P, \)
Kp, Mc+x), in the half-line {(x,y)/x>Kp, y=KMc} (i.e. boundary between domains D23 and D24) (point (f1(x), KMc)), and the image of the point (f1(x), KMc), in the segment {(x,y)=x>Kp, KMc<y<KMc} (i.e. boundary between domains D13 and D23) (point (Kp, f2(f1(x)))). From (4), we obtain the analytical expression of f1 and f2:

\[
\begin{align*}
    f_1(x) &= \frac{a^{24}x + K_Mc + b^{24}K_p}{a^{23} - x} \\
    f_2(x) &= \frac{-b^{23}x + K_p b^{23} - K_pK_{Mc} + a^{23}K_{Mc}}{a^{23} - x}
\end{align*}
\]

which are homographic functions. If f2(x)>KMc, the trajectory will firstly cross the boundary separating D23 and D24. Otherwise, the trajectory will firstly cross the boundary separating D23 and D24. We can thus derive the conditions on x0 to follow either the small or the large amplitude cycle:

\[
\begin{align*}
    f_2(f_1(x_0)) &> K_{Mc} \quad \text{to follow the small amplitude cycle} \\
    f_2(f_1(x_0)) &< K_{Mc} \quad \text{to follow the large amplitude cycle}
\end{align*}
\]

Since \( f_2 \circ f_1 \) is a continuous and strictly decreasing function, we can define its inverse function \( (f_2 \circ f_1)^{-1} \). This yields to the following conditions: 0<x0<x0 to follow the small amplitude cycle and x0>x0 to follow the large amplitude cycle, with \( x_0 = (f_2 \circ f_1)^{-1}(K_{Mc}) = (f_1^{-1} \circ f_2^{-1})(K_{Mc}) \). For the parameter values indicated in Fig.3, we have x0=0.077.

For the small amplitude cycle, we can define the image of:
- a point (Kp, x), (f1(x), KMc), for KMc<x<KMc and 0<f1(x)<Kp
- a point (x, KMc), (Kp, f2(x)), in the z axis for 0<x<KMc.

Similarly, for the large amplitude cycle, we can define the image of:
- a point (x, KMc), (f1(x), KMc), for x>Kp and f1(x)>Kp
- a point (x, KMc), (f1(x), Kp), for x>Kp and f1(x)>Kp
- a point (Kp, x), (f1(x), Kp), for 0<x<Kp and 0<f1(x)<Kp
- a point (x, KMc), (f1(x), KMc), for 0<x<Kp and 0<f1(x)<Kp
- a point (x, KMc), (f1(x), KMc), for 0<x<Kp and 0<f1(x)<Kp

From (4), we obtain the analytical expressions of \( f_i, i=3,\ldots,10 \). For example,

\[
    f_3(x) = \frac{a^{11}x - K_Mc a^{11} + K_Mc K_p - b^{13}K_p}{x - b^{11}}
\]

(see Abou-Jaoudé et al., 2010) for the analytical expressions of \( f_i, i=2,\ldots,10 \).

Therefore, the analytical expression of the first return map, F(x), from and to the z axis is:

\[
\begin{align*}
    F(x) &= (f_4 \circ f_3 \circ f_2 \circ f_1)(x) \quad \text{for } 0<x<x_0 \\
    F(x) &= (f_4 \circ f_6 \circ f_5 \circ f_4 \circ f_3 \circ f_1)(x) \quad \text{for } x>x_0
\end{align*}
\]

F is thus a composition of homographic functions. It is thus also a homographic function in each of the two intervals of definition. For the parameter values indicated in Fig.3, we obtain:

\[
\begin{align*}
    F(x) &= \frac{x}{0.23 + 16 \cdot x} \quad \text{for } 0<x<0.077 \\
    F(x) &= \frac{0.1 + 0.3 \cdot x}{1 + 2 \cdot 2 \cdot 0.1} \quad \text{for } x>0.077
\end{align*}
\]

From (5) and (6), we deduce that F admits two strictly positive fixed points, x1 and x2, and thus two periodic orbits, with x1~0.048 and x2~0.093 (Fig.4). Moreover, \( \frac{dF}{dx}(x=x_i) < 1, i=1,2 \). Therefore, the two periodic orbits are stable (Strogatz, 1994), proving the existence of birhythmicity.

Calculating the left-handed and the right-handed limit of F when x tends to x0, we get:

\[
\lim_{x \to x_0^-} F(x) = 0.054 \quad \text{and} \quad \lim_{x \to x_0^+} F(x) = 0.094
\]

Therefore, F admits a point of discontinuity at x=x0 (Fig.4).

We then investigated the role of the “tail”, which characterizes the large amplitude limit cycle, in the emergence of the birhythmicity (Fig.3). To do so, we suppressed this “tail”, while keeping the two embedded cycles in the transition graph, by adding a transition from domain D22 to domain D23 in the transition graph (Mc23 set to 1 nM). We then analyzed the fixed points and the continuity of the first return map G in the z axis for this modified Model 3. Our analysis shows that G has only one strictly positive fixed point at x=x1 and thus a single periodic orbit (see Abou-Jaoudé et al., 2010). Moreover, G is continuous and admits a non smooth point at x=x0 (Abou-Jaoudé et al., 2010). Therefore, the loss of the “tail” leads to the loss of the discontinuity point of F. This accompanies the loss of the fixed point, x=x0, corresponding to the large amplitude limit cycle. The “tail” is thus here necessary for the emergence of the birhythmic behavior.
4. CONCLUSION

The specific mechanisms leading to complex dynamical behavior such as birhythmicity can be studied by approximating a model into a more qualitative system and easier to interpret reduced model while preserving the properties of interest. Following this approach, we converted the sharp non-linearities of the OAK model into discontinuous step functions to reveal the phase space structure of the birhythmic behavior. This approximation coupled with a reduction of dimensionality, from a 3-dimensional to a 2-dimensional system by setting one variable as a constant in each domain of the phase space, allowed getting a reduced model from which basic features observed in the phase space characterizing birhythmicity have been extracted: (1) the presence of two embedded cycles in the transition graph of the piecewise affine models, (2) the presence of a “tail” in the orbit of the large amplitude limit cycle of long period.

The use of a first return map description further allowed proving the existence of birhythmicity in the framework of the 2-dimensional piecewise affine model and giving an interpretation of the role of these two features in the emergence of the birhythmic phenomenon. The analysis of the first return map showed that feature (1) was associated with a non smooth point of the return map whereas feature (2) further leads to a discontinuity in the return map, which is at the origin of the emergence of the birhythmic behavior. In general, on a methodological level, one major advantage of this approach is to greatly facilitate the computational analysis of the model. In this regard, this method could be implemented to analyze the dynamics of other mathematical models of biological rhythms showing high enough non-linearities.

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