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NOTES ON CIRCADIAN RHYTHM

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Abstract. We discuss a class of models arising in the study of circadian rhythm and the properties of the matrix equations providing the bifurcation points for a wide parameter class. In particular, we prove that the five dimensional system studied in the cited work of Gonze, Halloy, and Goldbeter can have only a simple Hopf bifurcation.

1. Introduction

Many instances of models for biological systems propose some type of feedback in a system of biochemical interactions. Such models are sought when the underlying phenomena has periodic structure, in that the feedback can give rise to periodicity. A well-known example is that of the circadian rhythm commonly found in animal species. That is, the fluctuation with a period of about 24 hours of many physiological attributes. Another, recently modeled phenomena is the segmentaton clock in development. In this case an underlying time periodic fluctuation gives rise to rhythmic additions to the growing body axis of many animals. Thus a spatial periodicity arises as a result of a time-periodic oscillation. The literature on periodicity in biology is immense. We do not propose to give an extended list, but merely cite Gonze, et al. [5], Goldbeter and Pourquié [3], and Goldbeter, et al. [4], leaving the reader to pursue the references in those papers.

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One type of model is based on a set of ordinary differential equations for the concentrations of chemical species in which the time rate of change of a particular product species depends instantaneously on the concentrations of the species itself and on the concentrations of the other components. A common feature found in many models is kinetics based on the Michaelis–Menten approximation (cf. [2]), giving rise to a system of nonlinear ordinary differential equations. A further feature that may be present in a system having concentrations $M, P_0, \ldots, P_n$, is that the rate of change of the level P_k depends only on P_k itself and on its "neighbours" $P_{(k-1)}$ and $P_{(k+1)}$, in a nonlinear fashion. Here one should imagine that $M \equiv P_{(-1)} \equiv P_{(n+1)}$ so that, not surprisingly, there is a closed chain of relations. In fact, the model in [5], presented by by the group at the Unité de Chronologie Théorique in Brussels (UCT), belongs to this class. Here we wish to demonstrate some properties that depend only on this chain structure and on a type of monotonicity that can be postulated about the chain.

A typical situation is one in which there are stable, stationary solutions, depending on one or more parameters of the system. Consider a system, written as

$$\frac{dS}{dt} = A(v, S) \tag{1.1}$$

in which the state S might represent the collection $M, P_0, \ldots, P_n$ and v stands for a parameter set $v_1, \ldots, v_k$.

The stationary solutions are the pairs $(\widehat{v}, \widehat{S})$ for which $A(\widehat{v}, \widehat{S}) = 0$. Those stationary pairs for which the Jacobian derivative $D_S A(v, S)$ has all of its eigenvalues strictly in the left half of the complex plane are stable and, while often of interest for homeostasis of a system, are not indicative of periodic structure. If one allows the parameter v to vary and, if at some pair $(\widehat{v}, \widehat{S})$, the Jacobian has an eigenvalue with real part equal to zero, then one has found a point of interest, a possible bifurcation point. If the eigenvalue on the imaginary axis has its imaginary part equal to zero, then one may expect a more elaborate collection of stationary solutions to exist nearby the pair $(\widehat{v}, \widehat{S})$ (cf. [1]). If, on the other hand, the eigenvalue on the imaginary axis has its imaginary part non-zero, then for a real valued system, the Jacobian must have a conjugate pair of eigenvalues $\alpha + i\beta$ and $\alpha - i\beta$, with $\beta \neq 0$. In general, the more elaborate structure of solutions nearby will include a periodic solution, arising from a Hopf bifurcation (cf. [1, Section 3.4]). This case is the one that one hopes for in modelling a natural system with known periodicity.

In addition to discussing properties of a system of any size, we will show that for the system in [5], in which there are five equations, the only possible bifurcations from the steady states of the model are simple Hopf bifurcations, presumed to give rise to periodic solutions. Naturally, the model in [5], solved numerically, exhibits periodic solutions and, in the parameter range studied, the periods are near 24 hours.

This report is a revised version of a part of the notes that the second author used for a lecture at a meeting in June 2006, dedicated to Prof. Dino Fortunato. The results have never been published, but we organize them here in the hope that they may be of interest to those examining biological models.

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2. Circadian models

The model in [5] treats the level M of mRNA and the concentrations P_0, P_1, P_2 and P of phosphorylated states of a protein PER. The explicit form for the equation (1.1) in this case is

$$
\begin{cases}
\dfrac{dM}{dt} = v_s \dfrac{K_I^n}{K_I^n + P^n} - v_m \dfrac{M}{K_m + M}, \\
\dfrac{dP_0}{dt} = k_s M - v_1 \dfrac{P_0}{K_1 + P_0} + v_2 \dfrac{P_1}{K_2 + P_1}, \\
\dfrac{dP_1}{dt} = v_1 \dfrac{P_0}{K_1 + P_0} - v_2 \dfrac{P_1}{K_2 + P_1} - v_3 \dfrac{P_1}{K_3 + P_1} + v_4 \dfrac{P_2}{K_4 + P_2}, \\
\dfrac{dP_2}{dt} = v_3 \dfrac{P_1}{K_3 + P_1} - v_4 \dfrac{P_2}{K_4 + P_2} - v_d \dfrac{P_2}{K_d + P_2} - k_1 P_2 + k_2 P, \\
\dfrac{dP}{dt} = k_1 P_2 - k_2 P.
\end{cases}
\tag{2.1}
$$

The unknown vector is $S = (M, P_0, P_1, P_2, P)$ and the parameter vector v consists of $(v_s, v_m, \ldots, K_I, K_m, \ldots, k_1, k_2)$, that is, all the symbols other than those constituting S. We shall assume that the parameters are positive, unless otherwise noted. In [5], n is a positive integer, taken to be 4 in numerical calculations.

The stationary equation $0 = A(v, S)$ is thus:

$$
\begin{cases}
0 = v_s \dfrac{K_I^n}{K_I^n + P^n} - v_m \dfrac{M}{K_m + M}, \\
0 = k_s M - v_1 \dfrac{P_0}{K_1 + P_0} + v_2 \dfrac{P_1}{K_2 + P_1}, \\
0 = v_1 \dfrac{P_0}{K_1 + P_0} - v_2 \dfrac{P_1}{K_2 + P_1} - v_3 \dfrac{P_1}{K_3 + P_1} + v_4 \dfrac{P_2}{K_4 + P_2}, \\
0 = v_3 \dfrac{P_1}{K_3 + P_1} - v_4 \dfrac{P_2}{K_4 + P_2} - v_d \dfrac{P_2}{K_d + P_2} - k_1 P_2 + k_2 P, \\
0 = k_1 P_2 - k_2 P.
\end{cases}
\tag{2.2}
$$

The “chain” structure of the system (2.1) makes it a simple matter to establish that there are stationary solutions for a range of parameters. To see this one can start with the last equation. Since $0 = k_1P_2 - k_2P$, this same expression is zero in the fourth equation, and so on. One arrives at a pair of equations $h_1(M,P) = 0$ and $h_2(M,P) = 0$ which can be solved graphically, being careful to note that one wants $M > 0$ and $P_i > 0$, limiting the allowable parameter ranges.

Suppose that $(\widehat{v},\widehat{S})$ is a stationary point, and consider the Jacobian determinant arising at that point. All the parameters in the collection v take positive values, with the result that all the elements in the Jacobian have signs. For example, the derivative of the first line in $A(v,S)$ (i.e. the right hand side of (2.1)) with respect to M will be negative and so can be written as $-a$ with a positive. Likewise the derivative with respect to P is negative and so can be denoted as $-\delta$ with δ positive. No other components of S are present in the first line. Continuing in this fashion one sees that the Jacobian determinant in a circadian system will be an essentially tridiagonal matrix (apart from the term $-\delta$ in the upper corner) with negative terms on the diagonal (apart from λ) and positive terms off the diagonal. The eigenvalue determinant for the system in [5] can thus be written

$$J_5(\lambda) = \begin{vmatrix} -a-\lambda & 0 & 0 & 0 & -\delta \\ k_s & -b-\lambda & c & 0 & 0 \\ 0 & b & -c-d-\lambda & e & 0 \\ 0 & 0 & d & -e-h-k_1-\lambda & k_2 \\ 0 & 0 & 0 & k_1 & -k_2-\lambda \end{vmatrix} \tag{2.3}$$

with all the symbols $a, b, c, d, e, \ldots$ appearing, being positive, again, apart from λ. Note that the parameter h appears only in the $(4,4)$ position. It is proportional to the parameter v_d used as a bifurcation parameter in [5].

3. A general Jacobian

Before examining the five by five determinant in the circadian model, we briefly look at some properties shared by such matrices of arbitrary size. For this discussion we merely assume the parameters in the string v are non-negative and explicitly assume positivity for some results. In fact, at closer examination, some results will hold when only a subset of the parameters is positive, but we do not pursue this.

The Jacobian determinant arising in the last section corresponded to a model with two phosphorylation steps. An analogous model with three phosphorylation steps would give rise to a determinant

$$J_6(\lambda)=\begin{vmatrix} -a-\lambda & 0 & 0 & 0 & 0 & -\delta \\ k_s & -b-\lambda & c & 0 & 0 & 0 \\ 0 & b & -c-d-\lambda & e & 0 & 0 \\ 0 & 0 & d & -e-f-\lambda & g & 0 \\ 0 & 0 & 0 & f & -g-h-k_1-\lambda & k_2 \\ 0 & 0 & 0 & 0 & k_1 & -k_2-\lambda \end{vmatrix}$$

with symbols other than λ positive and, with no phosphorylation steps, to a determinant

$$J_3(\lambda) = \begin{vmatrix} -a-\lambda & 0 & -\delta \\ k_s & -h-k_1-\lambda & k_2 \\ 0 & k_1 & -k_2-\lambda \end{vmatrix}.$$

We will refer to an n by n determinant of the type (J_6) as a *circadian determinant*, denote it by $J_n(\lambda)$, and qualify it, saying it has positive parameters, or non-negative parameters.

It is worth noting at this juncture that the differential equations with varying numbers of phosphorylation steps all possess periodic solutions in some parameter ranges, at least when solved numerically.

To examine some general structures present we begin by examining some strictly tridiagonal matrices. If we set $\delta = 0$ in the determinant $J_n(\lambda)$, then it becomes tridiagonal.

The sign properties of tridiagonal matrices may be well-know, but we include some results here, suited to our purposes. We let M_k denote a $k \times k$ tridiagonal determinant

$$\begin{vmatrix} -p_k-q_k-\lambda & p_{k-1} & \cdots & \cdots & 0 & 0 \\ q_k & -p_{k-1}-q_{k-1}-\lambda & p_{k-2} & \cdots & 0 & 0 \\ 0 & q_{k-1} & -p_{k-2}-q_{k-2}-\lambda & \cdots & 0 & 0 \\ \cdots & \cdots & q_{k-2} & p_3 & 0 & 0 \\ 0 & 0 & \cdots & -p_3-q_3-\lambda & p_2 & 0 \\ 0 & 0 & \cdots & q_3 & -p_2-q_2-\lambda & p_1 \\ 0 & 0 & \cdots & 0 & q_2 & -p_1-\lambda \end{vmatrix}$$

where $p_i \geq 0$, $q_i \geq 0$, $i = 1, \ldots, k$. It is understood that M_1 is merely the number $(-p_1 - \lambda)$ and for the purpose of induction we define $M_0 \equiv 1$.

LEMMA 3.1. *For $k \geq 1$ the expression $(-1)^k M_k(\lambda)$ is strictly positive if $\lambda > 0$ and is non-negative if $\lambda \geq 0$.*

PROOF. It will suffice to treat the case $\lambda > 0$. Let $N_k \equiv (-1)^k M_k$, for $k = 0, 1, \ldots$ We show that $N_k > 0$ for $k = 1, 2, \ldots$ It is clear for $N_1 = (-1)M_1 = p_1 + \lambda$. For $k \geq 2$ we have

$$M_k(\lambda) = (-p_k - q_k - \lambda)M_{k-1} - p_{k-1}q_k M_{k-2}. \tag{3.1}$$

From (3.1) we have

$$N_k(\lambda) = (p_k + q_k + \lambda)N_{k-1} - p_{k-1}q_k N_{k-2}$$

or, rewriting,

$$N_k(\lambda) - p_k N_{k-1} = \lambda N_{k-1} + q_k[N_{k-1} - p_{k-1}N_{k-2}].$$

Defining

$$R_k = N_k - p_k N_{k-1} \tag{3.2}$$

for $k \geq 1$, the identity above becomes

$$R_k = \lambda N_{k-1} + q_k R_{k-1}, \tag{3.3}$$

and is valid for $k \geq 2$. Recall, we are assuming $\lambda > 0$.

One readily sees that $R_1 = \lambda$ and hence, from (3.3) $R_2 = \lambda N_1 + q_2 R_1 > 0$. But then $N_2 > p_2 N_1 \geq 0$. Thus, from (3.3), $R_3 > 0$ and, a fortiori, $N_3 > p_3 N_2 > 0$. Inductively, both N_k and R_k are positive for all k. $\square$

Corollary 3.2. *For $\delta \geq 0$ and $k = 1, 2, \ldots$, the determinant $J_k(\lambda)$ has the alternation property*:

$$(-1)^k J_k \geq 0 \quad \text{for } \lambda \geq 0$$

and is positive for $\lambda > 0$.

Proof. The contribution of the term containing δ is easily seen to have the correct sign. Let $\delta = 0$ and focus on the parameter h. When $h = 0$ the form of J_k is that of M_k with $p_{k-1} = 0$ and with the awkward notation $k_1 = q_2$ and $k_2 = p_1$. As regards the dependence on h, exhibiting it now as $J_k(\lambda, h)$, it will suffice to show that

$$\frac{\partial}{\partial h}(-1)^k J_k > 0$$

when $\lambda > 0$ and the remaining parameters $a, b, \ldots$ are positive, so that the dependence is in the right direction. As for the h derivative, since h appears only in the entry indexed by $(k-1, k-1)$, imagining expanding the determinant using the penultimate column, one finds the sign of the h derivative of J_k is that of $(-1) * C$ where C is the cofactor of the $(k-1, k-1)$ entry. The cofactor, expanded, is $-k_2 - \lambda$ times a $(k-2)$ by $(k-2)$ determinant of the type treated in Lemma 3.1 and which has the sign of $(-1)^{(k-2)}$. The sign of interest is thus that of $(-1)^k(-1)^2(-1)^{(k-2)} > 0$. $\square$

An obvious corollary follows:

COROLLARY 3.3. *The k dimensional circadian determinant $(-1)^k J_k(\lambda) \geq 0$ for $\lambda \geq 0$ and is strictly positive for $\lambda \geq 0$ provided the product $\delta k_s bd \ldots k_1 > 0$.*

In fact, the last result holds even if certain parameters vanish. In Section 5 we shall see that $J_5(0) > 0$ provided that $abdhk_2 + \delta k_s bdk_1 > 0$. We again note that the bifurcation parameter v_d in [5] is proportional to our h appearing in the last expression.

LEMMA 3.4. *Suppose $p_i \geq 0$, $q_i \geq 0$, $i = 1, \ldots, k$. Then the determinant $M_k(\lambda)$ has all its roots on the non-positive real axis.*

PROOF. Again, the result will follow by induction. Suppose $\lambda = \alpha + i\beta$ with $\beta > 0$. From the relation

$$M_k(\lambda) = (-p_k - q_k - \lambda)M_{k-1}(\lambda) - p_{k-1}q_k M_{k-2}(\lambda),$$

assuming M_{k-1} does not vanish, we have,

$$\frac{(-1)^k M_k(\lambda)}{(-1)^{k-1}M_{k-1}(\lambda)} = p_k + q_k + \lambda - p_{k-1}q_k \frac{(-1)^{k-2}M_{k-2}(\lambda)}{(-1)^{k-1}M_{k-1}(\lambda)}.$$

Denoting the ratio on the left of the last equation by $z_k = a_k + ib_k$, the inductive relationship can be written

$$z_k = p_k + q_k + \lambda - p_{k-1}q_k \frac{1}{z_{k-1}}.$$

The imaginary part is then

$$b_k = \beta - p_{k-1}q_k \frac{-b_{k-1}}{a_{k-1}^2 + b_{k-1}^2} = \beta + p_{k-1}q_k \frac{b_{k-1}}{a_{k-1}^2 + b_{k-1}^2}.$$

Recall

$$M_2(\lambda) = (b + g + k_1 + \lambda)(k_2 + \lambda) - k_1 k_2 \quad \text{and} \quad (-1)M_1(\lambda) = (-1)(-k_2 - \lambda).$$

Clearly M_1 cannot vanish for $\beta > 0$ and hence

$$z_2 = a_2 + ib_2 = p_2 + g + \alpha + i\beta - \frac{q_2 q_1}{q_1 + \alpha + i\beta}.$$

The imaginary part is

$$b_2 = \beta + \frac{\beta k_2 k_1}{(k_2 + \alpha)^2 + (\beta)^2}$$

so $b_2 > 0$. By induction, for $\beta > 0$, $M_k(\alpha + i\beta)$ does not vanish for $k = 2, 3, \ldots$ and $b_k > 0$ for $k = 3, 4, \ldots$ so all roots are real. We have already eastablished that $M_k > 0$ for $\lambda > 0$, so the roots must be on the non-positive real axis.

In fact one can allow many of the parameters to vanish and still have the last result hold. □

Corollary 3.5. *A circadian determinant with positive parameters can never have zero as an eigenvalue and hence can have no bifurcation arising from a zero eigenvalue.*

4. The determinant J_5

Letting $q = \delta k_s bdk_1$ we now examine the behavior of the determinant

$$J_5(\lambda) = (-a-\lambda)M_4(\lambda) - q$$

which enters the analysis of bifurcation points for the five dimensional system (2.2).

A slightly tedious calculation shows that

$$M_4(\lambda) = \lambda^4 + m_3\lambda^3 + m_2\lambda^2 + m_1\lambda + m_0 \tag{4.1}$$

where

$$\begin{aligned}
m_3 &= b + c + d + e + k_1 + k_2 + h,\\
m_2 &= bd + ek_2 + be + ce + bk_1 + bk_2 + bh,\\
&\quad + ck_1 + ck_2 + ch + dk_1 + dk_2 + dh + hk_2,\\
m_1 &= bdk_1 + bdk_2 + bdh + bek_2 + cek_2 + bhk_2 + chk_2 + dhk_2,\\
m_0 &= bdhk_2.
\end{aligned}$$

Hence,

$$(\lambda + a)M_4(\lambda) = \lambda^5 + B_4\lambda^4 + B_3\lambda^3 + B_2\lambda^2 + B_1\lambda + B_0$$

where $B_4 = a + m_3$, $B_0 = abdhk_2$ and $B_k = am_k + m_{k-1}$, $k = 1, 2, 3$. From these, one easily shows that $(B_3)^2 - 4B_1 > 0$ and $B_4B_3 - B_2 > 0$. See the Appendix for more details.

For now we use J to denote J_5 and examine the behavior of the roots on parameters, putting aside any physical significance of parameter sizes for the moment. The roots of J are those of

$$(\lambda + a)M_4(\lambda) = \lambda^5 + B_4\lambda^4 + B_3\lambda^3 + B_2\lambda^2 + B_1\lambda + abdhk_2 + q. \tag{4.2}$$

Suppose we examine the case $a = b = d = 2$, $c = 0$, $e = 0$, $k_s = 1/4$, $k_1 = 1$, $h = 1$, $k_2 = 0$. Then (4.2) becomes $(-2-\lambda)^4(-\lambda) - q = 0$. It is relatively simple to show that, for q very large positive the roots of this last polynomial are asymptotic to the roots of $\lambda^5 = -q$ (cf. Appendix). A "plugin" shows that for $q > 0$ there can be no root of the form $-2 + i\beta$, $\beta \neq 0$ and thus for all $q > 0$, three roots stay to the left of this line and the two to the right of the line (one arising from $\lambda = 2$, at $q = 0$, the other from $\lambda = 0$) go into the complex, becoming asymptotic to two of the roots of $\lambda^5 = -q$ for large q. That is, a single pair crosses the imaginary axis with increasing q. For the case of general parameters, could there be a situation in which *two* pairs cross into

$$S^+ \equiv \{\lambda \mid \operatorname{Re}\lambda \geq 0\}?$$

We show that this cannot happen. The simple case provides more than a picture of what might happen. It provides a link to the general case via a homotopy.

We now fix a determinant of type (2.3) for the ensuing discussion and assume all the parameters are positive. Next, form a determinant of type (2.3) in which each original parameter (apart from δ) is replaced by a linear function of t connecting it to the special values given above. That is, for $0 \leq t \leq 1$ consider "parameters"

$$\begin{aligned} &a(t) = 1 - t + ta, \qquad &&b(t) = 1 - t + bt, \qquad &&d(t) = 1 - t + td, \\ &h(t) = 1 - t + th, &&k_1(t) = 1 - t + tk_1, && \\ &c(t) = tc, &&e(t) = et, &&k_2(t) = tk_2. \end{aligned} \tag{4.3}$$

The parameter which we have called q would now have the form

$$q(t) \equiv \delta k_s(t) \ldots k_1(t),$$

but we replace it by a non-negative parameter s that can be arbitrarily large, and which is independent of t. the resulting determinant will be denoted $J(\lambda, t, s) \equiv (-a(t) - \lambda)M_4(\lambda, t) - s$, showing the dependence on the the parameter t and on the parameter s.

For any fixed t, we have seen that with $s = 0$, but with the other parameters positive $J(\lambda)$ has all its five roots on the negative real axis (with parameters non-negative, roots are non-positive). As s increases from zero one can imagine the roots as forming five paths in the complex plane, parametrized by s. For the case $t = 0$, three paths remain in the left half plane and a pair of paths go off to infinity in the right half plane, as we have seen. We will examine the behavior of the roots as they depend upon s, putting aside any physical significance of parameter sizes for the moment. Allowing s to become extremely large and positive, one can show that the roots of J are asymtotically the roots of $\lambda^5 + s = 0$ and so have a pair in the right half of the complex plane with angle near $\pi/5$, as well as a root with angle near $3\pi/5$ (see the Appendix). Thus as s grows from zero, a pair of roots of $J(\lambda)$ must pass into the right half plane. However, for very large s there is precisely one pair in the right half plane. For $t = 1$, (the original determinant), could there be a value of s for which there are TWO pairs of conjugate eigenvalues in the the closed right half complex plane? The main result of this section states that this cannot happen.

The idea will be to use the parameter t as a homotopy parameter and show that, if there were two pairs of roots in the right half plane for $t = 1$, then one could lower the value of t to create a tangency condition on the imaginary axis, as a function of s, for some intermediate value of t. Or, there would have to be a double root on the axis. We show that neither case is allowable.

Theorem 4.1. *For a given set of positive parameters $a, b, \ldots$ the Jacobian determinant $J = J_5(\lambda)$* (2.1) *can have at most one pair of conjugate roots in the closed half-plane $S^+ \equiv \{\lambda \mid \operatorname{Re}\lambda \geq 0\}$.*

Corollary 4.2. *The only possibilites for bifurcation in the* 5*-dimensional circadian system* (2.3) *are simple Hopf bifurcations.*

We will require the following lemmas, for the second of which, we assume t is fixed and s varies. In both, for "imaginary axis" we exclude the value 0.

Lemma 4.3. *$J(\lambda)$ cannot have two distinct pairs of roots on the imaginary axis or a pair of multiplicity two on the imaginary axis.*

Proof. Suppose that the negative root is $-R$ and there are two pairs $\pm i\tau_1$, $\pm i\tau_2$, where we allow $\tau_1 = \tau_2$. Then $-J$ has the form

$$\begin{aligned} J(\lambda) &= (\lambda + R)(\lambda^2 + \tau_1^2)(\lambda^2 + \tau_2^2) \\ &= \lambda^5 + (\tau_1^2 + \tau_2^2)\lambda^3 + (\tau_1^2\tau_2^2)\lambda + R\lambda^4 + R(\tau_1^2 + \tau_2^2)\lambda^2 + R(\tau_1^2\tau_2^2). \end{aligned}$$

In particular, denoting the coefficient of λ^k by B_k, one has $B_4B_3 = B_2$ which, we have seen, is impossible. □

Lemma 4.4. *$J(\lambda)$ cannot have a pair of simple roots on the imaginary axis with the tangent, as a function of s, being vertical.*

Proof. Letting $\lambda(s) = \alpha(s) + i\beta(s)$ denote the root and s_0 the value for which $\alpha(s_0) = 0$ and $\beta(s_0) = \beta_0$, we have the identity in s: $J(\lambda(s)) = 0$. That is

$$\lambda^5(s) + B_4\lambda^4(s) + B_3\lambda^3(s) + B_2\lambda^2(s) + B_1\lambda + abdgk_2 + s = 0 \tag{4.4}$$

for s in a neighborhood of s_0. Taking the derivative in s (root simple gives differentiability) yields

$$(5\lambda^4(s) + 4B_4\lambda^3(s) + 3B_3\lambda^2(s) + 2B_2\lambda(s) + B_1)\lambda' = -1, \tag{4.5}$$

so the polynomial $5\lambda^4(s) + \ldots + B_1)$ must have real part zero at $i\beta_0$. Looking at the imaginary part of (4.4) and the real part of (4.5) one must have

$$\beta^5 - B_3\beta^3 + B_1\beta = 0, \qquad 5\beta^4 - 3B_3\beta^2 + B_1 = 0.$$

As $\beta > 0$, we can factor a β from the first, let $\beta^2 = \sigma$ and have

$$\sigma^2 - B_3\sigma + B_1 = 0, \qquad 5\sigma^2 - 3B_3\sigma + B_1 = 0.$$

Subtracting the two yields $\sigma = B_3/2$ as the only positive solution and this, put in the second equation, yields $4B_1 = B_3^2$, which is not possible for a system of the type considered.

Now define:

$$T = \{(t,s) \mid 0 \le t \le 1,\ s \ge 0,\ J(\lambda,t,s) \text{ has two pairs of roots in } S^+\},$$

where we include multiplicity; that is, a non-real, double root in S^+ counts for inclusion in T. Hence, having a pair $(t,s) \in T$ means there is precisely ONE root in $S^- = \{\lambda \mid \operatorname{Re}\lambda < 0\}$, the negative root. □

LEMMA 4.6. *T is compact.*

PROOF. It is shown in the Appendix that, uniformly in t, for large s there are precisely two roots in S^+, so T is bounded. To see that T is closed consider the complement T^c. It consists of the pairs (t,s) for which there are not two pairs of roots in S^+; that is, either there are no pairs in S^+ in which case there are 5 roots in S^- or there is one pair in S^+ in which case there are 3 roots in S^-. In either case, a small perturbation in the pair (t,s) will maintain the total multiplicity of roots in S^-. This can easily be shown using the resolvent of the matrix map defined by the matrix J (cf. Kato [6, Chapter II, 1.4]). □

PROOF OF THEOREM 4.1. If T is empty, then there is at most one pair of conjugate roots in S^+ for the original Jacobian. If T is not emply let

$$t_0 = \inf\{t \mid (t,s) \in T\}.$$

As T is compact, the inf is realized at some point (t_0, s_0) in T. It cannot be the case that $s_0 = 0$. First, by Lemma 3.4, all roots of $J(\lambda,t,0) = -(\lambda + a(t))M_4(\lambda,t)$ are on the non-positive real axis, so it suffices to exclude a multiple root at $\lambda = 0$. But from (4.1) and the explicit coefficients, one sees that

$$\left.\frac{\partial J(\lambda,t,0)}{\partial\lambda}\right|_{\lambda=0} = m_1(t) > 0 \quad \text{for all } t \in [0,1] \tag{4.6}$$

so such a root is simple. Let $\lambda_1(t_0,s_0)$, $\lambda_2(t_0,s_0)$ be the roots and suppose $\operatorname{Re}\lambda_1 \le \operatorname{Re}\lambda_2$ for $i = 1,2$. These may coincide if there is one root of multiplicity two. It cannot be the case that $\operatorname{Re}\lambda_i > 0$ for $i = 1,2$ for then, by continuity, there would be a $t_{00} < t_0$ yielding two pairs of conjugate roots for some s, again by use of a resolvent. As such, $\operatorname{Re}\lambda_1 = 0$ and the possibilites are:

(a) $0 = \operatorname{Re}\lambda_1 < \operatorname{Re}\lambda_2$,
(b) $0 = \operatorname{Re}\lambda_1 = \operatorname{Re}\lambda_2$, $\operatorname{Im}\lambda_1 \ne \operatorname{Im}\lambda_2$,
(c) $\lambda_1 = i\beta$ is a double root.

In case (a), the fact that t_0 is the minimum value of t for which there are two pairs of roots in T and T is compact implies that the eigenvalue $\lambda_1(t_0,s)$ as a function of s must be tangent to the imaginary axis at s_0. Otherwise we could change s slightly, entering the interior of S^+, and then find a point in T with a lower value of t. However, we have ruled out such a tangency in Lemma 4.4.

Cases (b) and (c) are dissallowed by Lemma 4.3 and hence T is empty. This proves the Theorem. $\square$

5. Appendix

5.1. Coefficients of J. From Section 4 we have:

$$(\lambda + a)M_4(\lambda) = \lambda^5 + B_4\lambda^4 + B_3\lambda^3 + B_2\lambda^2 + B_1\lambda + B_0$$

with

$$\begin{aligned}
B_4 &= a + b + c + d + e + k_1 + k_2 + h, \\
B_3 &= a(b + c + d + e + k_1 + k_2 + h) + e(k_2 + b + c) \\
&\quad + bd + hk_2 + [d + b + c][k_1 + k_2 + h], \\
B_2 &= aB_3 + (b + c)ek_2 + bd(k_1 + k_2 + h) + (b + c + d)hk_2, \\
B_1 &= a[(b + c)ek_2 + bd(k_1 + k_2 + h) + (b + c + d)hk_2] + bdhk_2, \\
B_0 &= abdhk_2.
\end{aligned}$$

Note that B_4 is a sum of single parameters; B_3, a sum of products, etc. This makes it a simple matter to establish that

$$(B_3)^2 - 4B_1 > 0 \quad \text{and} \quad B_4B_3 - B_2 > 0.$$

For the first claim we note that the expression for B_3 is

$$\begin{aligned}
&ab + ac + ad + ae + ak_1 + ak_2 + ag + ek_2 + eb + ec + bd + gk_2 \\
&\qquad\qquad + dk_1 + dk_2 + dg + bk_1 + bk_2 + bg + ck_1 + ck_2 + cg
\end{aligned}$$

and that for B_1 is

$$abek_2 + acek_2 + abdk_1 + abdk_2 + abdg + abgk_2 + acgk_2 + adgk_2 + bdgk_2.$$

Each term in B_1 is a product of two terms from B_3. For example $abek_2$ arises from ab times ek_2. The expression for $(B_3)^2 - 4B_1$ contains $(ab)^2 + 2abek_2 + (ek_2)^2 - 4abek_2$ which is $(ab - ek_2)^2 \geq 0$. Each of the eight terms in B_1 can be accounted for in this manner by a pair of terms among the 21 terms in B_3. Of course the 210 choices of pairs give many other positive products in the expression for B_3^2, so the inequality $(B_3)^2 - 4B_1 > 0$ is easily satified.

The inequality $B_4B_3 - B_2 > 0$ is even simpler. The term B_2 is $am_2 + m_1$ and consists of 22 positive terms. Each term occurring in B_2 is a product of a term in B_4 and a term in B_3, and is among the $8 \cdot 21 = 168$ terms in B_4B_3. As all terms involved are nonnegative, the inequality follows.

5.2. Roots of quintic polynomials. Consider a fixed set of postive parameters $a, b, c, \ldots, k_1, k_2$ coming from a circadian model, form the t dependent determinant using $a(t), b(t), \ldots$, and look at $J(\lambda, t, s) \equiv (-\lambda - a(t))M(\lambda, t) - s = 0$, where $s \geq 0$ is a free parameter, independent of t, with $0 \leq t \leq 1$. Consider large s and revert to the notation from (4.2). Let

$$Q(\lambda) \equiv \lambda^5 + B_4\lambda^4 + B_3\lambda^3 + B_2\lambda^2 + B_1\lambda + B_0 \tag{5.1}$$

so that one is discussing the quintic $Q(\lambda) = -s$. let $s = R^5$ and assume R is very large. Let us look at the possibility of roots on the boundary of the complex region Γ with radius $R\sigma$ for $1/2 < \sigma < 2$ and angle $\pi/2 < \theta < 7\pi/10$. For example, letting $\lambda = \sigma R$, a root on the segment with $\theta = \pi/2$, would have to satisfy

$$R^5\sigma^5(e^{i5\pi/2}) + B_4R^4\sigma^4(e^{i4\pi/2}) + \ldots + B_0 = -R^5$$

or, equivalently,

$$\sigma^5 i + (B_4/R) + \ldots + B_0/R^5 = -1$$

and for R large the terms with R in the denominator are too small to bridge the gap between $\sigma^5 i$ and -1. A similar argument shows there can be no root on the part of the boundary with angle $7\pi/10$. On the curved segment with radius $R/2$ the terms in the polynomial sum to less than needed and where the radius is $2R$, the term $(2R)^5$ dominates the sum of the remaining terms, making a root impossible.

Since we have fixed $a, b, \ldots$ for this argument and $t \in [0, 1]$, all coefficients B_k entering (5.1) are uniformly bounded and the lack of roots on the boundary of Γ will hold uniformly in t for s larger than a suitable s_b. Moreover, the same arguments hold for a simple homotopy between $(2+\lambda)^4\lambda = s$ and $\lambda^5 = s$. Again appealing to resolvent methods (cf. Kato [6]), since no roots of the polynomials can cross the boundary of Γ during the homotopies and since $\lambda^5 = s$ clearly has a root in Γ, so must all the polynomials for large s.

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