SUPPLEMENTARY NOTES FOR "DESIGN PRINCIPLES UNDERLYING CIRCADIAN CLOCKS"

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Throughout this note we refer to the main paper *Design principles underlying circadian clocks* by Rand, Shulgin, Salazar & Millar as **I**. A further preprint (3) that explains the mathematics behind our arguments is available from the website www.maths.warwick.ac.uk/ipcr/. The software tool mentioned below is also available from this site.

1. Approximating the singular value decomposition of M^*.

The linear mapping M^* relates the parameter change δk to the change $(\delta\tilde{\gamma}, \delta\tau)$ in the reparameterised limit cycle and period. It is therefore is from s-dimensional space to an infinite-dimensional space. To estimate the singular spectrum of M^* we must approximate it by a finite-dimensional operator i.e. by a matrix.

We do this by approximating the curve $\delta\tilde{\gamma}(t)$ by a vector. We fix a large integer N and approximate $\delta\tilde{\gamma} = \delta\tilde{\gamma}(t)$ by the vector $\overline{\delta\gamma}$ whose jth entry is $\delta\tilde{\gamma}(j/N)$ and approximate M^* by $M^N : \delta k \to (\overline{\delta\gamma}, \delta\tau)$ which is given by $\overline{\delta\gamma} = \sum_i \bar{\theta}_i \cdot \delta k_i$ where $\bar{\theta}_i$ is the vector whose jth entry is $\theta_i(j/N)$ and where

$$\theta_i(t) = \left.\frac{\partial}{\partial k_i}\right|_{k=k_0} \tilde{\gamma}_k(t).$$

This gives a matrix representation for $M^{(N)}$ in terms of the basis vectors $\bar{\theta}_i$. We have developed a software tool that rapidly calculates the quantities $\theta_i(t)$. Using the above results this enables us to compute M^* and its singular value decomposition to arbitrary accuracy.

2. Infinitesimal Response Curves

2.1. Unforced case i.e. DD or LL.

We consider the differential equation

$$\dot{y} = g(y, k) \tag{1}$$

where $y = (y_1, \ldots, y_n) \in R^n$ and $k = (k_1, \ldots, k_s)$ is the vector of parameters. We assume that (1) has a attracting periodic solution $y = \sigma_0(t)$ with period p_0 when $k = k_0$.

We consider how this solution changes as k is varied. To do this we fix a point $y_0 = g_0(0)$ on the periodic solution and consider a small $(n-1)$-dimensional hyperplane Σ which meets the periodic solution at the point y_0 and is transversal to the solution. For example, one could take Σ to be the plane normal to the tangent vector to the periodic solution at y_0. Near to y_0 there is a coordinate system $x = (x_1, \dots, x_n)$ such that (a) $x \in \Sigma$ if and only if $x_1 = 0$, (b) $y_0 = \underline{0} = (0, \dots, 0)$ and $g(y_0, k_0) = (1, 0, \dots, 0)$ in this coordinate system. Let the differential equation (1) in the new coordinate sysyem be given by

$$\dot{x} = f(x, k) \tag{2}$$

and the periodic orbit be given by $x = \gamma_0(t)$.

We consider solutions $Y(t) = Y(t, x_0, k)$ of the matrix variational equation

$$\dot{x} = f(x, k),\ \dot{Y} = A(t) \cdot Y,\ x(0) = x_0,\ Y(0) = I. \tag{3}$$

Here $Y(t) = Y(t, x_0, k)$ is a $n \times n$ matrix and $A(t) = A(t, x, k)$ is the Jacobian matrix of partial derivatives $(\partial f_i / \partial x_j)$ evaluated at x and k and the initial condition for this solution is that $Y(0)$ is the identity matrix I. If the matrix $Y(p_0)$ has exactly one eigenvalue equal to 1 then, for k near k_0, the system (3) has a unique periodic orbit $x = \gamma_k(t)$ near $x = \gamma_0(t)$.

The changes δQ caused to key output variables Q by variations δk in the parameters are linear functions of the change δp in the period $p = p(k)$ (not relevant for entrained forced systems) and the change $\delta\gamma(t)$ of the limit cycle. Let us write this relationship $\delta Q = L_Q \cdot (\delta\gamma, \delta p)$. Now consider

$$\begin{aligned} f_{k_i,\gamma,t}(s) \quad &= \quad -Y(t) \cdot \pi_2 \left(Y(p_0) - \mathrm{diag}[0, I_{n-1}]\right)^{-1} Y(p_0) Y(s)^{-1} b_i(s) \\ &\qquad + p_0^{-1} Y(t) \cdot \int_0^t Y(\sigma)^{-1} b_i(\sigma)\, d\sigma. \end{aligned} \tag{4}$$

and

$$f_{k_i,period}(s) = \pi_1 \left(Y(p_0) - \mathrm{diag}[0, I_{n-1}]\right)^{-1} Y(p_0) Y(s)^{-1} b_i(s)\, ds \tag{5}$$

where $p_0 = p(k_0)$. Here the vector $b_i(s)$ is $\partial f / \partial k_i$ evaluated at $y = \gamma_0(t)$ and $k = k_0$, $\pi_1(x_1, \dots, x_n) = x_1$ and $\pi_1(x_1, \dots, x_n) = (x_2, \dots, x_n)$

If k is changed by an amount $\delta k = (\delta k_1, \dots, \delta k_s)$ only when the phase is between s_1 and s_2 then

$$\delta\gamma(t) = \sum_i \delta k_i \cdot \int_{s_1}^{s_2} f_{k_i,\gamma,t}(s) ds + O(\|\delta k\|^2)$$

and

$$\delta p = \sum_i \delta k_i \cdot \int_{s_1}^{s_2} f_{k_i,period}(s) ds + O(\|\delta k\|^2).$$

Substituting these relationships into $\delta Q = L_Q \cdot (\delta\sigma, \delta p)$ gives the required relationship: if k_i is changed to $k_i + \delta k_i$ when the phase s is between s_1 and s_2 then

$$\delta Q = \sum_i \delta k_i \cdot \int_{s_1}^{s_2} f_{k_i,Q}(s) ds + O(\|\delta k\|^2).$$

The expressions in (4) and (5) can be very rapidly computed although accurate computation requires some careful numerical analysis to avoid the problems associate with the fact that $Y(t)^{-1}$ blows up as t gets large and even as $t \sim p$. The linear relation L_Q between $\delta\gamma$ and δp and δQ is easy to find. Therefore the IRCs can be rapidly computed.

2.2. **Entrained forced case e.g. LD.** This is more straightforword. We can ignore changes in the period since we are only concerned with entrained systems. Thus we only consider $f_{k_i,\gamma,t}(s)$ which in this case is given by

$$\begin{aligned} f_{k_i,\gamma,t}(s) &= -Y(t)\left(\pi_2 \left(Y(p_0) - I\right)^{-1} Y(p_0) Y(s)^{-1} b_i(s) \right. \\ &\quad \left. + p_0^{-1} \int_0^t Y(\sigma)^{-1} b(\sigma)\, d\sigma \right). \end{aligned}$$

where $\dot{y} = g(t, y, k)$ is the system under consideration and $Y(t) = Y(t, x_0, k)$ is the solution of the matrix variational equation

$$\dot{y} = g(t, y, k),\ \dot{Y} = A(t) \cdot Y,\ y(0) = x_0,\ Y(0) = I \tag{6}$$

with x_0 a point on the limit cycle. Here $Y(t) = Y(t, x_0, k)$ is a $n \times n$ matrix and $A(t) = A(t, x, k)$ is the Jacobian matrix of partial derivatives $(\partial g_i/\partial x_j)$ evaluated at t, x and k and the initial condition for this solution is that $Y(0)$ is the identity matrix I. The rest of the discussion proceeds as in the unforced case.

3. Output pathways amplitudes and phases

We consider a particular output pathway is driven by the molecular species whose level is given by $x_i(t)$. The change in the level of $x_i(t)$ at $t = t_0$ produced by a small change in the parameters can be calculated directly from the IRCs f_{k_i,Q_j} where $Q_j = x_i(t_0)$ via Equation (1) of the main paper **I**.

If we want to track the phase s of the minimum or maximum of $x_i(t)$ we can proceed as follows. The phase $s = s(k)$ satisfies $\dot{x}_i(s) = 0$ or equivalently $g_i(s, x(s), k_0) = 0$ where $\dot{x}_\ell = g_\ell(t, x, k)$, $\ell = 1, \ldots, n$ is the system under consideration. Differentiating this relationship with respect to k_j and solving for $\partial s/\partial k_j$

gives

$$\frac{\partial s}{\partial k_j} = \left(\sum_\ell \frac{\partial g_i}{\partial x_\ell} \cdot g_\ell \right)^{-1} \left(\frac{\partial g_i}{\partial t} + \sum_\ell \frac{\partial g_i}{\partial x_\ell} \cdot \left[\frac{\partial x_\ell}{\partial x_\ell^0} \frac{\partial x_\ell^0}{\partial k_j} + \frac{\partial x_\ell}{\partial k_j} \right] + \frac{\partial g_i}{\partial k_j} \right). \tag{7}$$

where x^0 is the point on the limit cycle that is the initial condition. In this expression derivatives of g_i and g_ℓ are evaluated at $x = x(s_0, k_0)$, $s = s_0$ and $k = k_0$, derivatives of x_ℓ are evaluated at $x = x^0$, $s = s_0$ and $k = k_0$, and the derivatives of x_ℓ^0 at $k = k_0$.

The derivatives of g are calculated directly. The derivatives $\partial x_\ell / \partial x_\ell^0$ are given by the matrix solution $Y(t)$ of either (3) or (6) above and those of $\partial x_\ell^0 / \partial k_j$ are given by integrating the IRC f_{k_ℓ, x^0} where x^0 is the point on the limit cycle at the starting phase.

4. Derivation of equation (3) of **I**.

We consider the situation where light of intensity I acts for a time interval of duration S from dawn to dusk. We suppose that this light acts by changing the parameter k_i to $k_i + \delta k_i(I)$. If the phase at dawn of the nth day is ϕ_n then at dusk it is $\phi_n + S + V(\phi_n)$ where

$$V(\phi) = -\delta k_i(I) \int_\phi^{S+\phi} f_{k_i, period}(t) dt \tag{8}$$

provided that the linear approximation is valid. Therefore at the end of the day the phase is given by

$$\phi_{n+1} = F(\phi_n) = \phi_n + V(\phi_n) + (L - p). \tag{9}$$

If there are multiple input pathways then one can combine them. For each parameter k_i affected by light one obtains a function V_i as in Equation (8) and then just adds them to get $V = \sum_i V_i$.

5. Mammalian model tracking dawn and dusk

We have added a new PER2-CRY2 loop to the mammalian model of reference (1). This has a different structure from the original PER-CRY loop. The structure of the new loop is based upon the PER-TIM loop of the model for *Drosophila* given in reference (2). For the new loop light activates transcription by increasing the maximum transcription rate. This rate is given by a Hill function which involves PER:CRY dimers as a negative transcription factor.

The different structure of the new loop is chosen because the original mammalian model of (1) tracks dusk and the new loop has been chosen to track dawn. It is necessary to mix systems tracking dawn and dusk because coupling two systems that track dusk would again give a system that tracks just dusk.

The new loop is linked into the original PER-CRY-CLOCK-BMAL model by the fact that PER2-CRY2 complexes with CLOCK-BMAL. Thus there is also an extra term in the equation for y_{14} the amount of CLOCK-BMAL. Otherwise, the equations for $y_1 \dots y_{16}$ are as in reference (1). The new equations are given below and the modification of the structure is shown in Figure 1. The term $f(t)$ represents forcing by light.

$$
\begin{aligned}
\text{(CLK:BMAL)} \quad \frac{dy_{14}}{dt} &= v_{3b}\frac{y_{14}}{(k_p+y_{14})}+v_{4b}\frac{y_{15}}{(k_{dp}+y_{15})} \\
&\quad +k_5y_{12}-k_6y_{14}-k_7y_{14}y_9+k_8y_{16}-k_{dn}y_{14} \\
&\quad +k_{84}\ y_{27}-k_{74}y_{14}y_{26} \\
\text{(per mRNA)} \quad \frac{dy_{17}}{dt} &= (\nu_{sp4}+amp_4f(t))\frac{k_{ip4}^{n_4}}{(y_{26}^{n_4}+k_{ip4}^{n_4})}-\nu_{mp4}\frac{y_{17}}{(y_{17}+k_{mp4})}-k_{d4}y_{17} \\
\text{(PER)} \quad \frac{dy_{18}}{dt} &= k_{sp4}y_{17}-\nu_{1p4}\frac{y_{18}}{(y_{18}+k_{1p4})}+\nu_{2p4}\frac{y_{19}}{(y_{19}+k_{2p4})}-k_{d4}y_{18} \\
\text{(PER-p1)} \quad \frac{dy_{19}}{dt} &= \nu_{1p4}\frac{y_{18}}{(y_{18}+k_{1p4})}-\nu_{2p4}\frac{y_{19}}{(y_{19}+k_{2p4})} \\
&\quad -\nu_{3p4}\frac{y_{19}}{(y_{19}+k_{3p4})}+\nu_{4p4}\frac{y_{20}}{(y_{20}+k_{4p4})}-k_{d4}y_{19} \\
\text{(PER-p2)} \quad \frac{dy_{20}}{dt} &= \nu_{3p4}\frac{y_{19}}{(y_{19}+k_{3p4})}-\nu_{4p4}\frac{y_{20}}{(y_{20}+k_{4p4})} \\
&\quad -k_{34}y_{20}y_{24}+k_{44}y_{25}-p\nu_{dp4}\frac{y_{20}}{(y_{20}+k_{dp4})}-k_{d4}y_{20} \\
\text{(cry mRNA)} \quad \frac{dy_{21}}{dt} &= \nu_{st4}\frac{k_{it4}^{n_4}}{(y_{26}^{n_4}+{k_{it4}}^{n_4})}-\nu_{mt4}\frac{y_{21}}{(y_{21}+k_{mt4})}-k_{d4}y_{21} \\
\text{(CRY)} \quad \frac{dy_{22}}{dt} &= k_{st4}y_{21}-\nu_{1t4}\frac{y_{22}}{(y_{22}+k_{1t4})}+\nu_{2t4}\frac{y_{23}}{(y_{23}+k_{2t4})}-k_{d4}y_{22} \\
\text{(CRY-p1)} \quad \frac{dy_{23}}{dt} &= \nu_{1t4}\frac{y_{22}}{(y_{22}+k_{1t4})}-\nu_{2t4}\frac{y_{23}}{(y_{23}+k_{2t4})} \\
&\quad -\nu_{3t4}\frac{y_{23}}{(y_{23}+k_{3t4})}+\nu_{4t4}\frac{y_{24}}{(y_{24}+k_{4t4})}-k_{d4}y_{23} \\
\text{(CRY-p2)} \quad \frac{dy_{24}}{dt} &= \nu_{3t4}\frac{y_{23}}{(y_{23}+k_{3t4})}-\nu_{4t4}\frac{y_{24}}{(y_{24}+k_{4t4})} \\
&\quad -k_{34}y_{20}y_{24}+k_{44}y_{25}-\nu_{dt4}\frac{y_{24}}{(y_{24}+k_{dp4})}-k_{d4}y_{24} \\
\text{(PER:CRY)} \quad \frac{dy_{25}}{dt} &= k_{34}y_{20}y_{24}-k_{44}y_{25}-k_{14}y_{25}+k_{24}y_{26}-k_{dc4}y_{25} \\
\text{(nucl. PER:CRY)} \quad \frac{dy_{26}}{dt} &= k_{14}y_{25}-k_{24}y_{26}-k_{dn4}y_{26} \\
&\quad +k_{84}y_{27}-k_{74}y_{14}y_{26} \\
\text{(P:CR:CL:B)} \frac{dy_{27}}{dt} &= -k_{84}y_{27}+k_{74}y_{14}y_{26}-\nu_{din}\frac{y_{27}}{(k_d+y_{27})}-k_{dn}y_{27};
\end{aligned}
$$

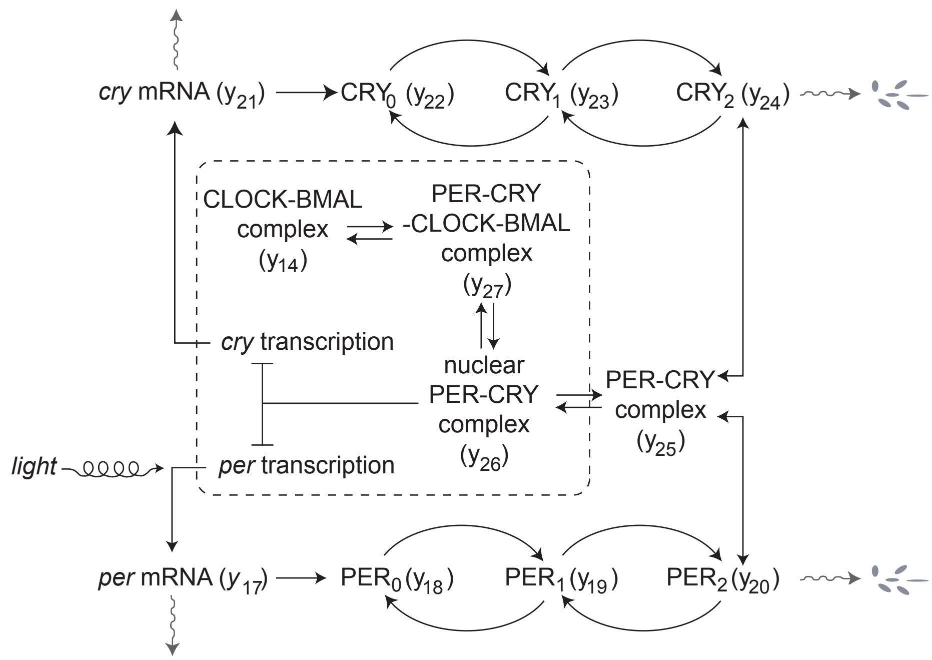

FIGURE 1. Schematic diagram showing the structure of the new loop and the way in which it is coupled into the original model.

parameter *value*	vsp4 0.7059	vst4 1.3594	vmp4 0.3873	vmt4 0.6862	pvdp4 0.6003	ksp4 1.0500	kst4 1.3000
k14 0.5000	k24 0.0931	k34 1.2284	k44 0.5466	kmp4 0.1387	kmt4 0.2000	kip4 1.6047	kit4 1.7371
kdp4 0.1937	kdt4 0.2000	kd4 0.0100	kdc4 0.0800	kdn4 0.0100	vdt4 2.0812	k1p4 2.5947	k1t4 1.9672
k2p4 2.0	k2t4 2.0	k3p4 1.9034	k3t4 2.3447	k4p4 2.0000	k4t4 2.0000	v1p4 7.7346	v1t4 7.3086
v2p4 1.0000	v2t4 1.0000	v3p4 6.3956	v3t4 7.6755	v4p4 1.0	v4t4 1.0	n4 3.3950	amp4 0.4
k74 0.05	k84 0.01						

TABLE 1. The values of the parameters used in the new loop.

References

[1] Leloup, J. C. and Goldbeter, A. (2003) Toward a detailed computational model for the mammalian circadian clock. *Proc. Natl. Acad. Sci. U. S. A.* **100** 7051-7056

[2] Leloup, J.C., Gonze, D., and Goldbeter, A. (1999). Limit cycle models for circadian rhythms based on transcriptional regulation in *Drosophila* and *Neurospora*. *J. Biol. Rhythms* **14**, 433-448.

[3] D. A. Rand, B. V. Shulgin, J. D. Salazar and A. J. Millar. Uncovering the design principles of circadian clocks: Mathematical analysis of flexibility and evolutionary goals. *Preprint 2004*

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