Delay-Differential Equations

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$$\frac{dN}{dt'} = rN[1 - \frac{N}{K}] \tag{1}$$

where r and K are the growth rate and carrying capacity and N is the population density. The independent time variable is t'. Equation 1 can be rewritten as

$$\frac{dN}{dt'} = R(N)N\tag{2}$$

where $R(N) = r(1 - \frac{N}{K})$ is a density-dependent growth rate and is a decreasing function of N. There are two steady states, $N_{\infty,1} = 0$ and $N_{\infty,2} = K$, but only the latter is stable (when r > 0). Thus, the population will tend to it's carrying capacity over time.

The logisite equation assumes that the organism's birth rate depends instantaneously on changes in population size. However, in many cases there should be a delay. For example, many organisms store nutrients, so won't notice immediately if the population grows too large for the available resources. In 1948 G. E. Hutchinson modified the logistic equation to incorporate a delay into the growth rate, so R(N) becomes $R(N(t' - \tau))$:

$$\frac{dN}{dt'} = rN[1 - \frac{N(t' - \tau)}{K}] \tag{3}$$

where τ is the **delay**. We can write this in dimensionless form by introducing the following changes of variable: $y \equiv \frac{N}{K}$ and $t \equiv \frac{t'}{\tau}$. A delay of τ is simply a delay of 1 in the new time variable. Also,

$$\frac{dN}{dt'} = \frac{dN}{dt}\frac{dt}{dt'} = \frac{1}{\tau}\frac{dN}{dt} \quad . \tag{4}$$

So in the dimensionless form,

$$\frac{dy}{dt} = r\tau y[1 - y(t-1)] \quad . \tag{5}$$

Define $\alpha \equiv r\tau$, so that the time delay is absorbed into the α parameter. Then Eq. 5 becomes

$$\frac{dy}{dt} = \alpha y [1 - y(t - 1)] \quad . \tag{6}$$

Now denote y(t-1) as y_{τ} ; it is a delayed variable. Then we finally arrive at the dimensionless delay-logistic equation,

$$\frac{dy}{dt} = \alpha y [1 - y_{\tau}] \quad . \tag{7}$$

Note that to specify initial conditions, one must specify the y value over the time interval [0, 1] (or $[0, \tau]$ for the dimensional form of the equation). Often this is done by picking y to be the same value over this entire interval, so $y = y_0$ for all $t \in [0, 1]$. In the examples below I use y = 2for $t \in [0, 1]$. Equation 7 has a single parameter, α , which is convenient for analysis. For the case $\alpha = 1$ the output is a damped oscillation that converges to 1. To find equilibria analytically we proceed as usual, but



Figure 1: Solution to delay-logistic equation with $\alpha = 1$.

also set $y_{\tau} = y$ since at equilibrium y does not change in time, so the delayed value of y is the same as the non-delayed value. From Eq. 7, the equilibria satisfy

$$0 = \alpha y_{\infty} (1 - y_{\infty}) \tag{8}$$

or $y_{\infty} = 0, 1$.

When α is increased to $\alpha = 2$ the damped oscillation becomes a sustained oscillation, with period of about 5 (Fig. 2). This indicates that a Hopf bifurcation has occurred for some value of α between 1 and 2.

We see from this example that a single delay-differential equation (DDE)



Figure 2: Solution to delayed-logistic equation with $\alpha = 2$.

is capable of producing periodic motion, in contrast to a single ordinary differential equation. This is because the single DDE is not 1-dimensional, but is actually **infinite dimensional**. This should be expected, since one must specify the value of y over an entire interval (an infinite number of points) to initialize the DDE.

So we see that by adding a delay one can induce oscillations. In this example, the delay was added into the term that provides negative feedback (the quadratic term), which was intentional; delayed negative feedback is well known to give rise to oscillations. As this example demonstrates, population models with a single species can give rise to oscillations, as long as there is delayed negative feedback.

Stability of the Linear Delay-Differential Equation

Local stability of DDEs is more challenging than for ordinary DEs, due to the infinite dimensionality of the system. As an example, we consider the simple linear delay-differential equation in dimensionless form

$$\frac{dy}{dt} = ay_{\tau} \tag{9}$$

where $y_{\tau} \equiv y(t-1)$. Note that the only equilibrium solution is $y_{\infty} = 0$.

Since Eq. 9 is linear, we try the exponential solution $y = Ce^{\lambda t}$, so that

$$\frac{dy}{dt} = C\lambda e^{\lambda t} \tag{10}$$

and also

$$ay_{\tau} = aCe^{\lambda(t-1)} = aCe^{\lambda t}e^{-\lambda} \tag{11}$$

so together,

$$\lambda - ae^{-\lambda} = 0 \tag{12}$$

is the **characteristic equation**. The solutions of this equation are called the eigenvalues, as in the case of a system of linear ordinary differential equations. For ODEs the equation is a polynomial and the fundamental theorem of algebra tells us how many roots (eigenvalues) to expect. For the linear DDE the characteristic equation is transcendental, so there is no theorem about number of roots (which could be countably infinite). For our example, we will consider real and complex solutions separately. First, **suppose that** λ **is real**. We can plot $z = \lambda$ and $z = ae^{-\lambda}$ and look for intersections.



Figure 3: For real λ and a > 0.

For a > 0 there is a single intersection at a positive λ . Thus, the solution $y = ce^{\lambda t}$ increases exponentially to infinity as $t \to \infty$, and the equilibrium $y_{\infty} = 0$ is **unstable**.

For a < 0 there may be 0, 1, or 2 intersections. There is a single intersection when the curves are tangent. At this point of tangency the curves have the same slope, and since the line has slope of 1, this means that at the point of tangency

$$\frac{d}{d\lambda}ae^{-\lambda} = 1\tag{13}$$

or

$$-ae^{-\lambda} = 1 \tag{14}$$



Figure 4: For real λ and three values of a < 0. There is a point of tangency for $a = a_c$ (green) and no real roots for $a < a_c$ (cyan).

so that

$$a = -e^{\lambda} \quad . \tag{15}$$

Since this point is on the curve $z = ae^{-\lambda}$, if we replace a, then z = -1. Since the intersection is on the line $z = \lambda$, $\lambda = -1$ also. Therefore, the tangency occurs (and there is a single intersection) when $a_c = -e^{-1}$.

For $a_c < a < 0$ there are two real negative eigenvalues. For $a = a_c = -e^{-1}$ there is a single negative eigenvalue ($\lambda = -1$). For $a < a_c$ there are no real eigenvalues.

By superposition, the solution to the linear DDE is the sum of exponential solutions. So, if $a \in [a_c, 0]$ the real eigenvalues are negative and so the associated exponential solutions decay to 0 over time. If $a < a_c$ there are no exponentially decaying or growing components to the solution. What about the components associated with complex eigenvalues? Suppose that λ is complex. Then substitute $\lambda = \lambda_r + i\lambda_i$ into the characteristic equation (Eq. 12). Then

$$\lambda_r + i\lambda_i = a \exp(-\lambda_r - i\lambda_i) \tag{16}$$

$$= a \exp(-\lambda_r) \exp(-i\lambda_i)$$
(17)

$$= a \exp(-\lambda_r) [\cos(-\lambda_i) + i \sin(-\lambda_i)]$$
(18)

$$= a \exp(-\lambda_r) \cos(\lambda_i) - ia \exp(-\lambda_r) \sin(\lambda_i)$$
(19)

SO

$$\lambda_r = a \exp(-\lambda_r) \cos(\lambda_i) \tag{20}$$

and

$$\lambda_i = -a \exp(-\lambda_r) \sin(\lambda_i) \tag{21}$$

and therefore

$$\frac{\lambda_r}{\lambda_i} = -\cot(\lambda_i) \quad . \tag{22}$$

Using these last two equations, we get parametric equations for λ_r and a with λ_i as a parameter:

$$\lambda_r = -\lambda_i \cot(\lambda_i) \tag{23}$$

$$a = -\frac{\lambda_i}{\exp(\lambda_i \cot(\lambda_i)) \sin \lambda_i} .$$
 (24)

Due to the periodicity of the trigonometric functions, many curves are traced out as λ_i is varied from $-\infty$ to ∞ , producing an infinite number of curves.



Figure 5: For complex λ there are an infinite number of solution curves (green). Also included is the solution curve for real λ (red).

The general solution is $y(t; a) = \sum c_n e^{\lambda_n t}$, where summation is over all values of λ_n for the given parameter a, and $y_{\infty} = 0$ is a **stable steady state** for values of a in which all eigenvalues have negative real parts. That is, for all values of a between the dashed line and $a = 0, a \in (a_{c2}, 0)$ in the figure. What is the value a_{c2} ? It is the value in which $\lambda_r = 0$ and $\lambda_i \neq 0$ (the eigenvalue is complex). From Eq. 20, if $\lambda_r = 0$, then

$$0 = a\cos(\lambda_i) \tag{25}$$

or

$$\lambda_i = \pm \frac{\pi}{2} + k\pi , \quad k \in \mathbb{Z}$$
(26)

and using Eq. 21,

$$\lambda_i = -a\sin(\lambda_i) \tag{27}$$

or

$$\lambda_i = \mp a \tag{28}$$

and thus

$$a = \mp \lambda_i \tag{29}$$

Equations 26 and 29 give the values of a where each of an infinite number of complex eigenvalues cross through $\lambda_r = 0$. We don't care about positive a values, since we know that in this case the real eigenvalue is positive, and hence $y_{\infty} = 0$ is unstable. For the negative values of a, the first crossing is at $\lambda_i = \frac{\pi}{2}$ and from Eq. 29, $a_{c2} = -\frac{\pi}{2}$. Therefore, $y_{\infty} = 0$ is stable for $a \in (-\frac{\pi}{2}, 0)$.

Linear Stability of the Delay-Logistic Equation

We now return to the delay-logistic equation,

$$\frac{dy}{dt} = \alpha y [1 - y_{\tau}] \quad . \tag{30}$$

In addition to the trivial steady state, this has a saturated steady state at $y_{\infty} = 1$. We will investigate the linear stability of this. To do this, introduce a small perturbation u from $y_{\infty} = 1$ and see if the phase point returns to the steady state. That is, let y = u + 1, or u = y - 1 and insert into the DDE,

$$\frac{du}{dt} = \alpha(u+1)(1 - (u_{\tau}+1))$$
(31)

$$= -\alpha(u+1)u_{\tau} \quad . \tag{32}$$

For small perturbations, $u + 1 \approx 1$, so

$$\frac{du}{dt} \approx -\alpha u_{\tau} \tag{33}$$

which is the linear DDE we just analyzed, with $a = -\alpha$. From this analysis, we know that the trivial solution is stable for $-\pi/2 < a < 0$, so $u_{\infty} = 0$ is stable for $0 < \alpha < \pi/2$. This means that a small perturbation away from $y_{\infty} = 1$ decays to 0 over time, and thus $y_{\infty} = 1$ is a stable steady state of the delay-logistic equation (Eq. 30) for $0 < \alpha < \pi/2$. At $\alpha = \pi/2$ this steady state loses stability when a complex eigenvalue crosses through the imaginary axis (the real part goes from negative to positive). Thus, there is a **Hopf bifurcation** at $\alpha = \pi/2$. For $\alpha > \pi/2$ the system has a stable periodic solution.



Figure 6: Solution to delay-logistic equation with $\alpha = 2$.

Periodic Breathing

Periodic breathing, also called Cheyne-Stokes breathing, is characterized by a few deep breaths followed by a few shallow breaths. In some cases, the deep breaths are followed by no breathing, a condition called sleep apnea. Periodic breathing often occurs in high altitudes during sleep, and is a symptom of altitude sickness. It also sometimes occurs in babies. During an apnea, blood oxygen levels and the heart pumping rate drop, causing the sleeper to wake up. Of course, this results in a bad night of sleep. In some extreme cases, death occurs during sleep. The leading hypothesis for periodic breathing is that it is due to an instability in the chemical control system for respiration. This hypothesis has gained support since it was first published, partly due to mathematical models (the first of which was published in 1940).

More recently (2000), a simple delay-differential equation model was published describing the dynamics of CO_2 concentration in the lungs (p). The equation is

$$V_L \frac{dp}{dt} = P - V - T \tag{34}$$

where P is CO₂ production, V is brain-controlled ventilation, and T is the transfer from lung to stores via the blood, and V_L is the lung volume.

 CO_2 production is a result of metabolism and is assumed to occur at a constant rate M. Ventilation is controlled by the brain stem, which monitors CO₂ levels in the blood. There is a delay between the measurement of the CO₂ level and the time at which ventilation takes place. The authors modeled this as $pV(p(t - \tau))$, where V is a ventilation function. The transfer to stores does not involve the brain, so it occurs without a delay and is described by the function T(p). As we shall see, the details of the ventilation and transfer functions are not needed to understand the instability hypothesis for periodic breathing. We now have

$$V_L \frac{dp}{dt} = M - pV(p_{\tau}) - T(p) \quad .$$
(35)

We assume that there is a steady state solution, p^* , that represents normal ventilation. Then we consider a small perturbation from p^* , $u = p - p^*$, and see if the system returns to rest:

$$V_L \frac{du}{dt} = M - (u + p^*) V(u_\tau + p^*) - T(u + p^*) \quad . \tag{36}$$

Now do a Taylor approximation, treating u and u_{τ} as separate variables,

$$f(u, u_{\tau}) \approx f(0, 0) + \frac{\partial f}{\partial u}(0, 0)u + \frac{\partial f}{\partial u_{\tau}}(0, 0)u_{\tau}$$
(37)

where

$$f(u, u_{\tau}) = M - (u + p^*)V(u_{\tau} + p^*) - T(u + p^*)$$
(38)

and f(0,0) = 0 since (0,0) is the steady state.

$$\frac{\partial f}{\partial u} = -V(u_{\tau} + p^*) - T'(u + p^*)$$
(39)

$$\frac{\partial f}{\partial u}(0,0) = -V(p^*) - T'(p^*) \tag{40}$$

$$\frac{\partial f}{\partial u_{\tau}}(0,0) = -p^* V'(p^*) \tag{41}$$

SO

$$V_L \frac{du}{dt} \approx -[V(p^*) + T'(p^*)]u - p^* V'(p^*)u_\tau \quad .$$
(42)

This tells us that all we need to solve the linearized DDE is $V(p^*)$, $T'(p^*)$, and $V'(p^*)$ (we don't need to know the complete V and T functions). Fortunately, these things are measurable, and have been measured. $V(p^*)$ is the mean ventilation; $T'(p^*) = \beta Q$ where β is the solubility of CO₂ in blood and Q is the cardiac output; and $V'(p^*)$ is the chemoreflex gain. To solve, insert $u = ce^{\lambda t}$ into Eq. 42,

$$V_L c \lambda e^{\lambda t} = -c [V(p^*) + T'(p^*)] e^{\lambda t} - c e^{\lambda t} e^{-\lambda \tau} p^* V'(p^*)$$
(43)

or

$$V_L \lambda = -[V(p^*) + T'(p^*)] - e^{-\lambda \tau} p^* V'(p^*)$$
(44)

and thus

$$\lambda + \frac{V(p^*) + T'(p^*)}{V_L} + \frac{e^{-\lambda\tau} p^* V'(p^*)}{V_L} = 0$$
(45)

and so the characteristic equation is

$$\lambda \tau + x + y e^{-\lambda \tau} = 0 \tag{46}$$

with the τ introduced to make the algebra easier later. Here

$$x \equiv \frac{[V(p^*) + T'(p^*)]\tau}{V_L}$$
(47)

$$y \equiv \frac{p^* V'(p^*)\tau}{V_L} . \tag{48}$$

Periodic breathing, by hypothesis, begins when the steady state CO_2 concentration in the lungs loses stability via Hopf bifurcation. So we look for solutions to the characteristic equation of the form $\lambda = i\omega$:

$$i\omega\tau + x + ye^{-i\omega\tau} = 0 \tag{49}$$

or

$$i\omega\tau + x + y[\cos(\omega\tau) - i\sin(\omega\tau)] = 0 \quad . \tag{50}$$

For the real part,

$$x + y\cos(\omega\tau) = 0 \tag{51}$$

and for the imaginary part,

$$\omega \tau - y \sin(\omega \tau) = 0 \quad . \tag{52}$$

From Eq. 52,

$$y = \frac{\omega\tau}{\sin(\omega\tau)} \tag{53}$$

and inserting this into Eq. 51,

$$x = -\omega\tau \cot(\omega\tau) \quad . \tag{54}$$

These latter two equations are parametric equations for x and y, with $\omega \tau$ as the parameter. We can plot the curve described by the equations over some range of $\omega \tau$. Here we use $0.5\pi < \omega \tau < 0.9\pi$.



Figure 7: Periodic breathing occurs for (x, y) above the Hopf curve.

This curve is almost linear. One can then rewrite Eq. 42 in terms of x and y, yielding

$$\frac{du}{dt} = -\frac{x}{\tau}u - \frac{y}{\tau}u_{\tau} \quad . \tag{55}$$

If (x, y) values below the Hopf curve are inserted into Eq. 55 and if the equation is solved numerically, we see that below the Hopf curve the equilibrium solution u = 0 is stable while above the curve it is unstable and there are stable oscillations. That is, periodic breathing occurs for (x, y) above the Hopf curve (Fig. 7).



Figure 8: Clinical data showing patients exhibiting periodic breathing (filled) and those exhibiting normal breathing (open). From Francis et al., Circulation, 102:2214-2221, 2000.

Figure 8 shows clinical data from patients exhibiting periodic breathing (filled squares) and the observed x and y values for these patients. It also shows individuals not exhibiting periodic breathing (open symbols). As predicted from the model, patients showing periodic breathing have x and y values that place them above the Hopf curves, where oscillations are expected. The cycle time for these patients is 1.2 ± 0.2 min. We can use this oscillation period along with the Hopf bifurcation theorem to deduce a value for the delay τ that must exist for our model to reproduce the experimental data. Let T denote the oscillation period, and then by the

Hopf bifurcation theorem,

$$T = \frac{2\pi}{\omega} \tag{56}$$

SO

$$\frac{T}{\tau} = \frac{2\pi}{\omega\tau} \tag{57}$$

so that

$$\tau = \frac{\omega\tau}{2\pi}T\tag{58}$$

$$= \frac{1.2}{2\pi} (\omega \tau) \quad . \tag{59}$$

Using the range $0.5\pi < \omega\tau < 0.9\pi$ that we used to make Fig. 7, the range of delays that will make the model range of periods match those from the clinical data is $0.3 < \tau < 0.5$ (min), or $18 < \tau < 30$ (sec). That is, the model predicts that when periodic breathing occurs there is a delay of somewhere between 18 and 30 seconds between the time at which the brain stem measures CO₂ level in the blood and the time that ventilation occurs.

Ultradian Rhythms in Glucocorticoid Secretion

Another biological example of oscillations induced by time delays is in the endocrine system. Glucocorticoids are hormones released by the adrenal gland during periods of stress. The glucocorticoid released in humans is cortisol, while that released in rats and mice (where virtually all studies are done) is corticosterone. These are key stress hormones that act on the cardiovascular, cognitive, metabolic, and immunological state of the animal. Under normal conditions, there is a daily or circadian rhythm in the release of glucocorticoids (which we'll call "CORT"), with high release during waking hours and low release during sleep. Superimposed on this slow rhythm is a faster rhythm with period of about 1 hour; this faster type of rhythm is called an ultradian rhythm. The amplitude of the ultradian rhythm is modulated by the circadian rhythm (Fig. 9). It has been demonstrated that the response of the animal to a stressor varies with the phase of the ultradian rhythm at which the stressor occurs.

The structure of the "stress axis" is shown in Fig. 10. The hypothalamus is the region of the brain that regulates hormone secretion from the various endocrine glands. It does this by acting on the pituitary gland, which contains five types of hormone-secreting cells, including pituitary corticotrophs. A neurohormone called corticotropin-releasing hormone (CRH) is released from the paraventricular nucleus (PVN) of the hypothalamus



Figure 9: Measurements of blood corticosterone in two male rats. The bar indicates the dark phase. From Walker et al., 2010.

and it stimulates the corticotrophs to secrete adrenocorticotropic hormone (ACTH). This travels through the blood to the adrenal gland, where it acts on cells of the adrenal cortex to secrete CORT into the blood. This hormone feeds back onto neurons of the PVN and on the corticotrophs to inhibit them. However, the CORT is not stored in the adrenal cells, but must be synthesized in response to ACTH. This introduces a time delay between the release of ACTH and the release of CORT.

The circadian oscillations in CORT are due to input from the body's central clock, a region of the brain called the **suprachiasmatic nucleus (SCN)**. This acts on neurons of the PVN so that the release of CRH undergoes a daily rhythm, causing daily rhythms in ACTH and CORT. (The **central nervous system (CNS)** also acts on the hypothalamus



Figure 10: Illustration of the key components of the stress axis.

to regulate hormone secretion.) It was originally thought that ultradian rhythms in CORT were also due to ultradian rhythms in CRH. However, the lab of Stafford Lightman has shown that ultradian rhythms in CORT can be produced when CRH levels are relatively constant. How can this happen? To answer this, he collaborated with mathematician John Terry and his student Jamie Walker to build and ultimately test a mathematical model.

This model includes a variable for the ACTH concentration (a), glucocorticoid receptor availability in the corticotrophs (r), and CORT (c). The equations are:

$$\frac{da}{dt} = \frac{p_1}{1 + p_2 rc} - p_3 a \tag{60}$$

$$\frac{dr}{dt} = \frac{(cr)^2}{p_4 + (cr)^2} + p_5 - p_6 r \tag{61}$$

$$\frac{dc}{dt} = a_{\tau} - c \tag{62}$$

where the p_j are parameters and $a_{\tau} = a(t - \tau)$. (The equations are in non-dimensional form.) The first equation reflects the inhibition of ACTH release when CORT binds to a receptor in the corticotrophs. The second equation reflects the increase in CORT receptor transcription and membrane insertion that occurs in response to CORT. The last equation desribes the delayed synthesis of CORT.

Simulations with this model were done with XPPAUT to produce Fig. 11. Initially the CRH input is low (parameter p_1 is small). In this case there are no oscillations. At the closed arrow the CRH concentration is increased and oscillations begin. In these oscillations the ACTH concentration (red) lead the CORT concentration (black), as has been observed experimentally. The oscillations are terminated, however, when the time delay τ is too small (open arrow). This demonstrates that oscillations only occur for appropriate values of the input parameter (CRH) and the time delay. It is a common feature with models involving explicit time delays that oscillations only occur when the time delay is sufficiently large.



Figure 11: Model simulation showing effects of CRH and the time delay on CORT and ACTH dynamics.

Another property of systems with an explicit time delay is that when oscillations occur their period depends critically on the value of the delay τ . Larger time delays give larger periods, but the oscillation periods are significantly greater than the time delay. Figure 12 is a 2-parameter bifurcation diagram that illustrates the region of parameter space where oscillations occur, and the periods of the oscillations. Oscillations only occur when the time delay is sufficiently large, regardless of the amount of CRH drive. Thus, the model predicts that the mechanism for ultradian CORT oscillations depends critically on the fact that CORT must be synthesized (not stored) in the adrenal cortex following ACTH stimulation.



Figure 12: Two-parameter bifurcation diagram showing the region in parameter space where ultradian oscillations occur. The parameters are CRH drive (p_1) and adrenal delay (τ) . From Walker et al., 2010.

The dependence of oscillation period on the time delay is the focus of Fig. 13. Note that the interval of CRH drive (p_1) over which oscillations are produced increases when the time delay is increased. In addition, the period increases with the time delay, and is always much greater than the time delay.



Figure 13: Period of the ultradian oscillation for several values of the time delay $\tau = T_{lag}$. From Walker et al., 2010.

Circadian Oscillations

The timing of behaviors of plants and animals is determined largely by cycles of night and day, giving an approximate 24-hour periodicity in the environment. Oscillations with a 24-hour period are called **circadian** oscillations. Circadian oscillations in hormones have evolved to adapt to the 24-hour rhythmicity of the environment. These oscillations are endogenous to the body, since they persist in an artificial environment of contstant dark or constant light. This can be demonstrated behaviorally by wheel running, which hamsters do when they are awake and when they have a wheel to run on (Fig. 14). The figure also shows actograms from heterozygous and homozygous *tau* mutant hamsters, which exhibit activity periods of 22 hrs and 20 hrs, respectively. The discovery of this mutation, by Martin Ralph and Michael Menaker in 1988, demonstrated that a mutation on a single allele can have a very large influence on the circadian period. It was eventually determined to be on the gene for casein kinase 1 epsilon.



Figure 14: Actogram of wheel running by hamsters in constant darkness. The period of activity in this free-running environment is 24.2 hours (top). The middle actogram shows wheel running in a heterzygous tau mutant, while the lower actogram shows that of a homozygous tau mutant hamster. Both mutants exhibit faster rhythms in activity. From Liu et al., 1997.

As mentioned in the last section, circadian oscillations in the body are driven by the suprachiasmatic nucleus (SCN), a small nucleus in the hypothalamus (Fig. 15). This is one of the few brain regions that receives direct input from the retina, via the retino-hypothalamic tract of neural fibers. This provides the input needed to entrain the SCN oscillator to the light/dark cycle.



Figure 15: Nuclei of the hypothalamus.

How do the neurons in the SCN produce a daily rhythm? Is it a product of a neural network within the SCN? To check this one can take the SCN from a hamster and dissociate the neurons in culture, so that they are not interconnected. Figure 16 demonstrates the firing rate of a single dissociated SCN neuron over time. A circadian rhythm in firing rate is obvious, demonstrating that network interations among SNC neurons are not necessary for circadian rhythm generation.

How do the ion channels in the membrane of the SCN neuron produce a circadian rhythm? The most obvious mechanism is through oscillations in gene transcription, which produces one or more gene product that varies



Figure 16: Firing frequency recorded from dissociated hamster SCN neurons over time. The lower two recordings are from heterozygous and homozygous tau mutant hamster. The homozygous mutant exhibits a shorter period. Liu et al., 1997.

ion channel activity on a daily basis. Figure 17 shows one such periodic gene called the period gene (per) whose product is the period protein (PER). These mRNA measurments come from mice sacrificed at different time points over a number of days. During the first day the mice are exposed to a 12/12 light/dark cycle, while they are in constant darkness after that (the dashed bar represents a period of time that would have corresponded to a light period, were the mouse not in constant darkness).

There is a clear rhythm in the abundance of *per* mRNA, with high levels during the light phase and low levels during the dark phase.

It is interesting that similar rhythms in *per* mRNA are observed in peripheral tissue such as the liver, kidney, and pancreas (Fig. 18). However, this rhythm dies out over time in peripheral tissue, while it persists in SCN explants. It is thought that periodic input from the SCN is needed to synchronize the oscillations in peripheral cells.



Figure 17: Daily rhythm in expression of the period gene in the SCN that continues in constant darkness. Measurement made using in situ hybridization. From Shigeyoshi et al., 1997.

What is the mechanism for the circadian rhythm in *per* gene expression?



Figure 18: Oscillations in *per* gene expression in peripheral tissue. Measurement made using a *per*-driven luciferase reporter. From Repport and Weaver (2002).

This has been the focus of a great deal of research in molecular biology for the past couple of decades, and now a great deal is known. The essence of the mechanism is given by a simple DDE model published by Scheper et al. (1999). There is a single variable for *per* mRNA concentration (m) and one for "effective" *PER* protein (p), which is the protein after phosphorylation. It is known that phosphorylated *PER* can enter the cell's nucleus and act as a transcriptional repressor of it's own gene! That is, m is inhibited by p. Thus, the m DDE is

$$\frac{dm}{dt} = \frac{r_m}{1+p^2} - q_m m$$

where r_m is mRNA production rate constant and q_m is an mRNA degradation constant. The effective protein p is produced by m (via mRNA translation), followed by several phosphorlyation steps. Finally, for p to repress m it must translocate across the nuclear membrane. This all takes time, so the production of p from m is delayed. The p DDE is

$$\frac{dp}{dt} = r_p m_\tau^3 - q_p p$$

where r_p is the effective protein production rate constant, q_p is the protein degradation rate constant, and $m_{\tau} = m(t - \tau)$.

As in the last example, the system has delayed negative feedback. It is therefore expected that oscillations can be produced with a sufficiently long time delay. In this case, we want the oscillations to have a period of about 24 hours. It turns out that this occurs if the time delay $\tau = 4$ hours, as shown in Fig. 19. We see that the mRNA level peaks before the protein level, which has been observed in the *Drosophola* (house fly) eye, where many studies on circadian gene expression have been performed.

The rhythm shown in Fig. 19 is called a **free-running rhythm**, since there is no input from a light source. In the animal, this input is provided by the retina via the retino-hypothalamic tract. This input acts to entrain



Figure 19: Period mRNA and effective protein time courses in the DDE model. The free-running period is 24.6 hr.

the oscillator to the light/dark cycle. In the Scheper model, best results were achieved when light input was modeled as an increase in the protein degradation rate, q_p . Figure 20 shows that when short pulses of doubled q_p are applied at a period of exactly 24 hr the oscillator is entrained to this period (rather than the free-running period of 24.6 hr). In fact, when the stimulus period is as long as 28 hr the oscillator is still entrained. A similar sort of entrainment process is thought to occur in animals.

Probably the most well-known model for circadian gene expression was developed by Albert Goldbeter in 1995. This is instructive since



Figure 20: Entrainment of the Scheper model occurs when it is subjected to short periodic pulses in which q_p is doubled.

the rhythm also depends on a time delay between transcription of PER mRNA, but this time delay is implicit in the system, rather than explicit as in the Scheper model. The system diagram is shown in Fig. 21. There are variables for the period mRNA (M), unphosphorlyated protein (P_0) , singly phosphorylated protein (P_1) , doubly phosphorylated protein (P_2) , and nuclear doubly phosphorylated protein (P_n) . The implicit delay is

due to the time required for the protein to be phosphorylated and then transported from the cytosol into the nucleus.



Figure 21: System diagram for the Goldbeter model of circadian period gene expression. See Goldbeter, 1995.

Since the delay is implicit, the equations are ordinary differential equations, rather than delay-differential equations. The per mRNA equation is:

$$\frac{dM}{dt} = v_s \frac{K_I}{K_I + P_N} - v_m \frac{M}{K_m + M}$$

where the repression of transcription by nuclear PER is achieved by putting P_n in the denominator of the transcription term. The second term reflects degradation of the mRNA. (Note that translation does not come at the expense of mRNA.) The P_0 ODE incorporates translation that creates protein, phosphorylation that converts P_0 into P_1 through the action of a kinase, and dephosphorylation that converts P_1 into P_0 through the action of a phosphatase:

$$\frac{dP_0}{dt} = k_s M - v_1 \frac{P_0}{K_1 + P_0} + v_2 \frac{P_1}{K_2 + P_1}$$

The other phosphorylated states are described by:

$$\frac{dP_1}{dt} = v_1 \frac{P_0}{K_1 + P_0} - v_2 \frac{P_1}{K_2 + P_1} - v_3 \frac{P_1}{K_3 + P_1} + v_4 \frac{P_2}{K_4 + P_2}$$
$$\frac{dP_2}{dt} = v_3 \frac{P_1}{K_3 + P_1} - v_4 \frac{P_2}{K_4 + P_2} - k_1 P_2 + k_2 P_n - v_d \frac{P_2}{K_d + P_2}$$

where the linear terms reflect transport into and out of the nucleus. Note that protein degradation only occurs in the doubly phosphorylated state, and indeed this is the target of proteases that chew up protein. The P_0 and P_1 proteins are also targets, but to a much lesser extent.

Once the period protein is doubly phosphorylated it can move into the nucleus and repress period transcription. The nuclear period protein equation is:

$$\frac{dP_n}{dt} = k_1 P_2 - k_2 P_n$$

This is the complete Goldbeter model, with 5 variables and 17 parameters. Since there is no explicit time delay τ , one has a harder time adjusting parameters to make the system produce a circadian rhythm. Goldbeter found a good combination of parameter values for this. In subsequent years he extended the model, and looked at questions such as entrainment by light pulses.

One advantage that the Goldbeter model has over the Scheper model is that the factors yielding the time delay (phosphorylation and nuclear transport) are in the model, while they are treated as a black-box time delay in the Scheper model. Thus, the Goldbeter model is more mechanistic. It also has more variables that can, in principle, but compared with actual data. However, the Scheper model is more intuitive, and clearly captures the idea that circadian oscillations in gene expression are due to delayed negative feedback. As is typically the case, there is no "right" model: both models have something to contribute to our understanding of the biological system.

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