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AJP - Endo 290:573-582, 2006. doi:10.1152/ajpendo.00428.2005

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Am J Physiol Endocrinol Metab 290: E573–E582, 2006;
doi:10.1152/ajpendo.00428.2005.

A mathematical model for the mating-induced prolactin rhythm of female rats

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Submitted 7 September 2005; accepted in final form 27 October 2005

Bertram, Richard, Marcel Egli, Natalia Toporikova, and Marc E. Freeman. A mathematical model for the mating-induced prolactin rhythm of female rats. *Am J Physiol Endocrinol Metab* 290: E573–E582, 2006; doi:10.1152/ajpendo.00428.2005.—For the first 10 days of pregnancy and the first 12 days of pseudopregnancy, the secretion of prolactin (PRL) from pituitary lactotrophs is rhythmic, with two surges/day. This rhythm can also be triggered by bolus injection of oxytocin (OT). We describe a mathematical model for the initiation, maintenance, and termination of the OT-induced PRL rhythm. In our model, the mechanism for this circadian rhythm is mutual interaction between lactotrophs and neuroendocrine dopamine (DA) neurons. This rhythm is, under normal lighting conditions, entrained by the suprachiasmatic nucleus (SCN) but persists in the absence of input from the SCN. We postulate that OT injection triggers the rhythm by activating a population of bistable hypothalamic neurons that innervate and inhibit DA neurons. The bistable nature of these neurons allows them to act as a memory device, maintaining the rhythm long after OT has been cleared from the blood. The mechanism for this memory device and the arguments supporting it are detailed with computer simulations. Finally, we consider potential targets for a rhythm-terminating factor and make predictions that may be used to determine which mechanism is operational in terminating the OT- or mating-induced PRL rhythm.

oxytocin; vasoactive intestinal polypeptide; dopamine; lactotrophs

DURING THE FIRST 10 DAYS of pregnancy in rats, the hormone prolactin (PRL) is secreted by pituitary lactotrophs in a circadian fashion, consisting of morning and afternoon surges (47). The increase in PRL secretion is vital for maintaining the structural integrity of the corpus luteum that, in turn, is responsible for progesterone synthesis (46). It has been shown (23, 24) that the PRL rhythm can be initiated in ovariectomized rats by a copulatory stimulus, which demonstrates that the rhythm does not require ovarian hormones and suggests that it is instead generated by interaction between the hypothalamus and the PRL-secreting pituitary lactotrophs. The goal of this study was to develop a mathematical model for the mechanism of the PRL rhythm, as well as the mechanisms for its initiation and termination.

PRL secretion is regulated by several hypothalamic nuclei. The primary regulatory hormone is dopamine (DA), which is secreted by neuronal populations within the arcuate and periventricular nuclei (37). DA is an inhibiting factor (4, 44) that ensures that PRL secretion is maintained at a low level, unless it is triggered by an appropriate stimulus, such as a copulatory stimulus (CS). Tuberoinfundibular (TIDA) neurons of the arcuate nucleus supply most of the DA via hypophysial portal vessels. Two other neuronal populations, tuberohypophysial and periventricular hypothalamic dopaminergic neurons, also contribute to the DA regulation of PRL secretion (22).

The feedback between DA neurons and lactotrophs is bidirectional because PRL stimulates DA synthesis and secretion from hypothalamic DA neurons (15, 28, 39). This stimulatory action is through a signal transduction pathway that involves gene expression within the DA neurons (22). Hence, the action of PRL on hypothalamic DA neurons involves a significant time delay that we postulate to be crucial for the PRL rhythm. Indeed, we suggest that the mechanism for the mating-induced PRL rhythm is the mutual interaction between DA neurons and lactotrophs and that the time delay of PRL stimulation is the primary factor setting the period of the free-running oscillation.

Because the PRL rhythm is a daily rhythm, it seems likely that the suprachiasmatic nucleus (SCN) of the hypothalamus plays some role. The SCN has been described as the master circadian clock of the brain because many of its constituent neurons exhibit daily rhythms in electrical activity, even when they are isolated in cell culture (31). The endogenous SCN rhythm is entrained by the light-dark regimen via input through the retinohypothalamic tract (48). Circadian rhythms in the SCN are transmitted to other regions of the body via the neurotransmitters arginine vasopressin and vasoactive intestinal polypeptide (VIP). It has been shown that VIPergic efferent connections from the SCN innervate DA neurons of the arcuate nucleus (25), and that VIP inhibits DA neuron activity (26). This pathway provides the means through which the SCN circadian clock can entrain the mating-induced PRL rhythm. Indeed, under normal lighting conditions, VIP neurons of the SCN have elevated activity in the early morning, providing time-of-day information to the target cells (26, 43, 45).

The CS that triggers the PRL secretory rhythm is very brief compared with the duration of the rhythm (10–12 days). This raises the question of the mechanism for the "memory" that maintains the PRL rhythm long after the triggering event (51). The fact that the PRL rhythm is maintained in ovariectomized CS animals demonstrates that the memory is not due to ovarian hormones but is instead encoded in the hypothalamus or preoptic area (12) or the pituitary. It has been shown that OT is released into the circulation in response to CS in sheep (32), rats (42), and humans (21). Recently, we (18) have shown that a single bolus injection of OT can induce a circadian PRL rhythm that is very similar to the secretory pattern of pregnant/pseudopregnant rats. Therefore, it seems plausible that the transient increase in OT levels that is induced by CS is the trigger for the PRL rhythm. However, it is not clear whether the OT levels remain elevated for the duration of PRL rhythm. When the PRL rhythm was initiated by OT bolus injection, OT

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in the blood returned to basal levels within 1 day of the injection, and yet the PRL rhythm continued (18). So although a rise in OT may be the trigger for the PRL rhythm, it does not seem to be the memory that maintains the rhythm.

Here, we investigated several mechanisms for the memory, and on the basis of model simulations we postulate that a population of OT-sensing hypothalamic neurons serves as the memory. Although we have not yet identified these neurons, we describe the properties that should characterize their behavior.

In pregnant rats, the PRL rhythm is thought to be terminated by placental lactogen (PL), a PRL-like hormone that is secreted by the placenta (2, 53). This hormone binds to PRL receptors on TIDA neurons and stimulates their activity. The increased DA secretion then inhibits lactotrophs and terminates the PRL rhythm (8, 35). The mechanism of rhythm termination in ovariectomized rats is unknown, so in principle it may be mediated by either activation of DA neurons (as occurs in pregnant rats) or direct inhibition of lactotrophs. We investigated the viability of both mechanisms.

BASIC MODEL EQUATIONS

We begin by developing a mathematical model for the activity levels of hypothalamic DA neurons and pituitary lactotrophs. This is a phenomenological model, where one variable is used to describe the activity level of an entire cell population. Such models have been used in prior investigations of the neuroendocrine system (11, 36). We let PRL represent the activity level of lactotrophs and DA represent the activity level of hypothalamic DA neurons and begin with a description of the differential equation for the time dynamics of PRL.

Isolated lactotrophs, removed from hypothalamic regulatory factors, exhibit a high level of tonic activity (3). We represent the tonic (endogenous) drive by the factor T_p. In vivo, the lactotrophs are subject to inhibition by DA neurons. We incorporate this inhibitory action by dividing T_p by $1 + k_d\mathrm{DA}^2$. The parameter k_d reflects the strength of the inhibition. The first term of the PRL differential equation is then

$$\frac{T_p}{1 + k_d\mathrm{DA}^2} \qquad (1)$$

This has a maximum value of T_p (no DA inhibition) and declines to 0 as DA concentration is increased. The second term in the PRL equation provides first-order clearance, so that if PRL is elevated it will tend to return to a basal level over time. This term is $-q\mathrm{PRL}$, where q is the clearance rate. The PRL equation is then

$$\frac{d\mathrm{PRL}}{dt} = \frac{T_p}{1 + k_d\mathrm{DA}^2} - q\mathrm{PRL} \qquad (2)$$

The DA neurons of female rats are also tonically active, and this tonic activity is responsible for keeping PRL levels low in the unstimulated animal (13). The tonic activation is represented by the factor T_d. As described earlier, PRL has been shown to stimulate DA activity. We expect that this stimulation would be delayed for up to several hours because the PRL effect on DA neurons involves gene expression (30, 41). In support of this, one study revealed a significant time delay between the subcutaneous injection of PRL and the increased DA release from TIDA neurons (16). We introduce the delayed stimulatory action of PRL by multiplying T_d by $1 + k_p\mathrm{PRL}_\tau^2$. The notation PRL_τ represents the PRL concentration at time $t - \tau$, where τ is a time delay. The factor k_p is the strength of the PRL feedback. As with the PRL equation, we include a first-order clearance term, $-q\mathrm{DA}$. For simplicity, we use the same clearance rate q. Using different values for the clearance rates has no qualitative effect on the results presented later. The DA equation is then

$$\frac{d\mathrm{DA}}{dt} = T_d(1 + k_p\mathrm{PRL}_\tau^2) - q\mathrm{DA} \qquad (3)$$

Parameter values for this phenomenological model were set to produce the PRL rhythm and are given in Table 1. The equations were solved numerically using the CVODE algorithm implemented in the XPPAUT software package (19).

RESULTS

DA-PRL feedback loop is the mechanism for PRL rhythm. Our hypothesis is that the DA-PRL feedback loop is the mechanism for the mating-induced PRL rhythm. To determine whether this mechanism is viable, we performed a numerical simulation with the DA-PRL model. The rhythm was initiated and maintained by including a factor $-T_d$ in *Eq. 3*. This lowers DA to a sufficiently low level so that the PRL rhythm can be initiated and maintained. Mechanisms for initiation and maintenance of the rhythm are investigated later. In the model simulation shown in Fig. 1, the DA activity is low in the early morning (midnight is *time 0*), so PRL rises for several hours (Fig. 1*B*, 1). The increase in PRL leads to a delayed activation of DA neurons, which causes a decline in PRL (Fig. 1*B*, 2). Thus PRL peaks while DA is beginning to rise. DA continues to increase, even as PRL falls, because it is responding to the PRL stimulatory drive from several hours earlier. Eventually, DA begins to decline because of the low PRL drive (Fig. 1*B*, 3). PRL begins to rise as the inhibitory DA level falls (Fig. 1*B*, 4), leading to a second PRL peak, the afternoon PRL surge (Fig. 1*B*, 5).

From this simulation, we see that the DA-PRL feedback loop is capable of producing a PRL rhythm with two peaks/day. One key prediction of this rhythmogenic mechanism is that lactotroph and DA neuron activity levels are out of phase with one another. That is, DA activity peaks after the morning PRL peak and before the afternoon PRL peak. Such a phase relationship has been demonstrated in pregnant rats (35, 40), ovariectomized CS rats (38), and OT-injected ovariectomized rats (18). A second prediction of the model is that, with the DA-PRL loop alone, the PRL peaks are of equal amplitude. That is, the morning and afternoon PRL surges are identical, contrary to data showing that the morning surge is typically larger than the afternoon surge (10, 29). Thus we conclude that

Table 1. *Model parameters*

$T_p = 6$	$k_d = 1$	$q = 0.5$	$T_d = 10$
$k_p = 0.03$	$\tau = 3$ h	$r_v = 2$	$v_o = 0.01$
$p_{inj} = 1000$	$v_{on} = 0.0002$	$r_n = 0.2$	$r_H = 5, 15$
$\tau_H = 40$ h			

See text for definitions.

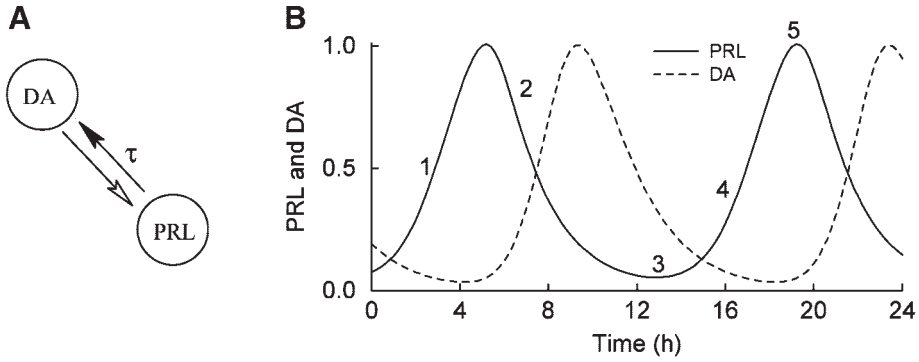

Fig. 1. Rhythm generated by the dopamine-prolactin (DA-PRL) feedback loop. *A*: schematic diagram illustrating network interactions in the DA-PRL subsystem. Inhibitory action of DA on PRL is illustrated by open arrow, and delayed stimulatory action of PRL on DA by closed arrow with label τ. *B*: there are two identical PRL surges/day (midnight is *time 0*). PRL curve is labeled to correspond to text. DA peak occurs between PRL surges. Curves here and in all other figures are normalized to maximum values. To initiate and maintain oscillation, a factor, $-T_d$, was added to *Eq. 3* to counteract tonic drive to DA neurons.

another component is needed in the model to account for the different amplitudes of the PRL surges.

The most important prediction of the DA-PRL model is that the PRL rhythm is an endogenous rhythm generated by interactions between the arcuate nucleus and the pituitary gland. It can be produced without any rhythmic input from the SCN or other hypothalamic nuclei. In particular, the rhythm should persist in the absence of photic cues. We note that the period of the oscillation (the free-running period) is ~28 h, which, of course, is longer than the period of the actual PRL rhythm. The reduction of the period to 24 h is discussed in the next section.

A key parameter in the DA-PRL model is the time delay, τ. In Fig. 1, $\tau = 3$ h, and the desired PRL rhythm, with two surges/day, is produced. The free-running oscillation period is shorter for smaller time delays and larger for larger delays (Fig. 2). In fact, if $\tau \leq 1$ h, the rhythm stops entirely and the system is at a steady state, with PRL elevated above its basal level. This is because the PRL stimulation of DA neurons is so rapid that PRL has little time to increase before it is suppressed by DA. Thus PRL and DA equilibrate rather than oscillate. Therefore, the model predicts that the delay in the PRL stimulation of DA neurons must be >1 h. We use $\tau = 3$ h as a somewhat extreme case to demonstrate in the next section how input from the SCN can drastically reduce the period of the PRL rhythm.

SCN entrains PRL rhythm and enhances morning surge. As described earlier, VIP neurons of the SCN innervate DA neurons of the arcuate nucleus and likely provide time-of-day information. We now add this pathway to the DA-PRL model through the parameter VIP, which represents the activity level of VIP neurons in the SCN. Because under normal lighting conditions the activity of these neurons is high during the morning (26, 43, 45), we model VIP as a square pulse that is elevated for 3 h in the morning and 0 at all other times. It has been shown (26) that the activity of DA neurons is inhibited by VIP released from neurons of the SCN. This inhibitory action is included in the differential equation for DA by adding the term r_vVIP·DA. The influence of VIP is incorporated in a multiplicative manner so that VIP only inhibits active cells, ensuring that DA does not become negative. The DA differential equation becomes

$$\frac{d\mathrm{DA}}{dt} = T_d(1 + k_p\mathrm{PRL}_\tau^2) - q\mathrm{DA} - r_v\mathrm{VIP}\cdot\mathrm{DA} \qquad (4)$$

A simulation of the PRL rhythm with the updated model is shown in Fig. 3*B*. Figure 3*C* shows that, when VIP is elevated (dashed line), the DA activity is reduced (solid line). The reduction in DA stimulates the model lactotrophs, advancing the occurrence of the first PRL surge. Thus the VIP input ensures that the first PRL surge occurs at the same time each morning, entraining the PRL rhythm to a 24-h period. Without VIP, the first PRL surge would drift, because the free-running DA-PRL rhythm has a period >24 h, with our choice of the delay τ. In addition to entraining the PRL rhythm to a circadian period, VIP breaks the symmetry of the two PRL surges. Because VIP inhibits DA before the morning PRL surge, but not the afternoon PRL surge, the magnitude of the morning surge is greater than that of the afternoon surge (Fig. 3*B*). This asymmetry is typically what is observed in the PRL rhythm (10, 29).

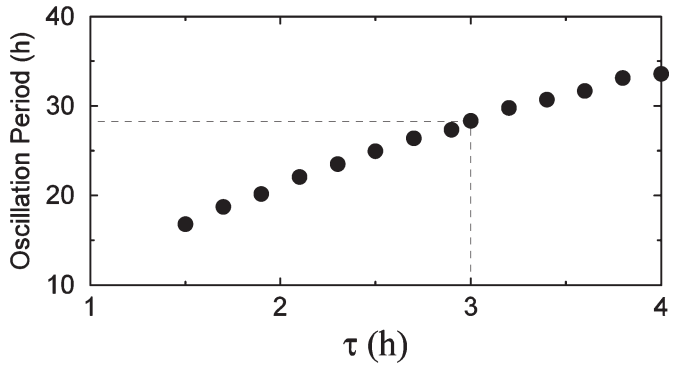

Fig. 2. Free-running oscillation period (2 PRL surges) depends on time delay of stimulatory action of PRL on DA neurons. Default time delay chosen in this paper, 3 h, produces free-running oscillation with a period of ~28 h.

OT injection triggers PRL rhythm, but how? Data suggesting that CS induces a rise in the OT level in the circulation led us to investigate the effects of a bolus injection of OT into ovariectomized rats. We (18) found that OT injection initiated a circadian PRL rhythm that is similar to that induced by CS. However, the OT in the circulation returned to basal levels within 1 day after the injection, whereas the PRL rhythm continued for at least another 4 days. Thus, whereas OT injection can trigger the PRL rhythm, it does not appear to be responsible for its maintenance.

What is the mechanism for the memory that maintains the PRL rhythm long after the triggering event? One possibility is that the OT acts directly on lactotrophs. This is reasonable because we (17) have shown that OT stimulates cultured lactotrophs. To investigate this possibility, we add another term to *Eq. 2*, reflecting the stimulatory action of OT on PRL. We use a simple linear function, v_oOT, that increases

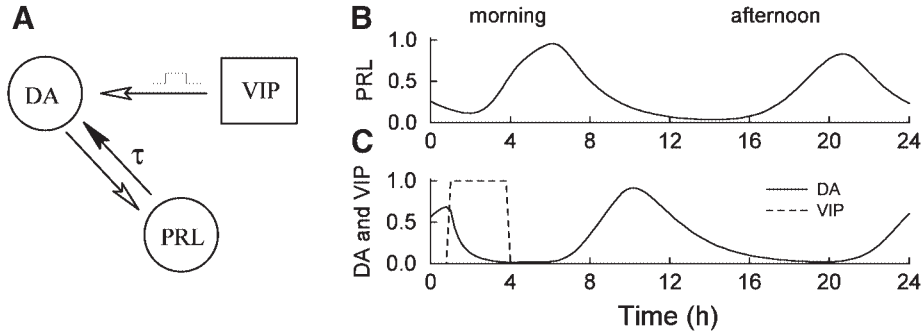

Fig. 3. *A*: schematic diagram illustrating network interactions. Vasoactive intestinal polypeptide (VIP) is treated as step function that is elevated for 3 h early each morning. *B*: VIP entrains PRL rhythm so that first surge occurs at the same time each day. VIP also causes magnitude of morning PRL surge to be enhanced relative to afternoon surge. *C*: VIP inhibits DA in the morning, advancing time of occurrence of the first PRL surge. DA peak is out of phase with PRL peaks. A factor, $-T_d$, was added to *Eq. 3* to counteract tonic drive to DA neurons, allowing rhythm to be produced.

with OT with a slope of v_o. The PRL differential equation then becomes

$$\frac{d\text{PRL}}{dt} = \frac{T_p}{1 + k_d\text{DA}^2} - q\text{PRL} + v_o\text{OT} \qquad (5)$$

To describe a bolus injection of OT and its clearance from the blood, we developed an OT differential equation. The first term simulates the OT injection, $p_{inj}I$. The factor $I = 1$ for a 2-h period immediately after an OT injection. At all other times, $I = 0$. The parameter p_{inj} reflects the magnitude of the injection. The second term in the OT differential equation describes the clearance of OT from the blood, and for simplicity the clearance rate (q) is assumed to be the same as the clearance rates for PRL and DA. Thus the differential equation for the OT level in the circulation is

$$\frac{d\text{OT}}{dt} = p_{inj}I - q\text{OT} \qquad (6)$$

Figure 4 shows the result of bolus OT injection with this model. (Note that the time scale is now days rather than hours.) Before injection, PRL is low, with only small upward inflections due to the daily pulsing of VIP (which reduces the activity of the DA neurons). The OT injection (Fig. 4*D*) results in a large deflection in both OT and PRL, and later in DA. However, OT returns to its baseline level by the end of the 1st day, and so, too, does PRL. Thus, with the direct stimulatory action of OT on lactotrophs alone, bolus OT injection produces only a transient rise in PRL. The circadian PRL rhythm is not induced.

One possibility is that the OT activates a long-lasting process in lactotrophs that persists for many days. This could be due to the actions of G proteins or some other second-messenger system. If OT does activate a long-lasting factor in lactotrophs that acts to stimulate the cells over a period of many days, can this induce the circadian PRL rhythm? To test this, we added a stimulatory factor (SF) term to the PRL equation

$$\frac{d\text{PRL}}{dt} = \frac{T_p}{1 + k_d\text{DA}^2} - q\text{PRL} + v_o\text{OT} + \text{SF} \qquad (7)$$

The value of SF is 0 before OT injection and 2 after OT injection, ensuring that the model lactotrophs are activated for a period of many days. Simulations with this model are shown in Fig.5. The PRL activity level is clearly elevated after OT injection, even though OT returns to basal levels within 1 day after the injection. However, the PRL rhythm is not initiated. This is true regardless of the magnitude of the stimulatory factor SF (not shown). The model, therefore, suggests that the ability of a bolus OT injection to start a circadian PRL rhythm is not due to the direct action of OT on lactotrophs alone.

Bistable OT-sensing neurons are a potential memory device. Figures 4 and 5 demonstrate that the stimulatory action of OT on lactotrophs is not sufficient to trigger the circadian PRL rhythm. Perhaps the DA neurons are subject to a long-lasting inhibition as a result of OT injection? If so, this action is unlikely direct, because OT receptors have not been detected on TIDA neurons (27). Therefore, we con-

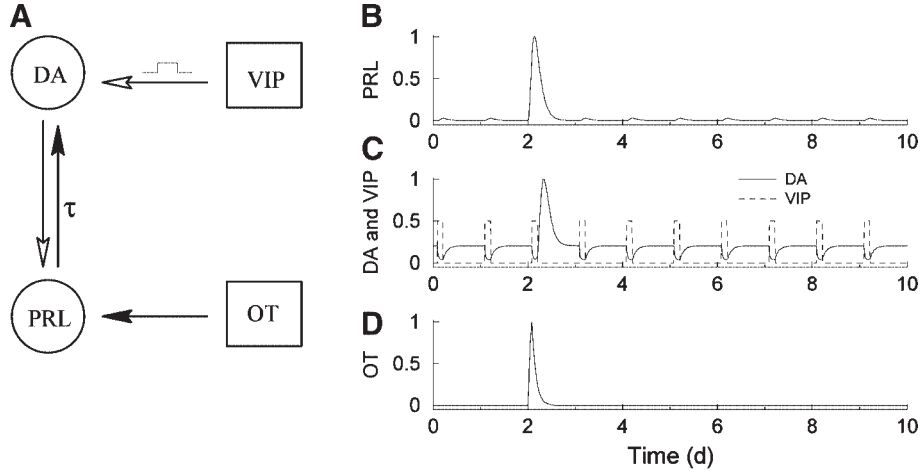

Fig. 4. *A*: diagram of a model in which oxytocin (OT) stimulates lactotrophs. *B*: PRL responds to simulated OT injection, but the response lasts only as long as elevated OT level. Circadian PRL rhythm is not generated. *C*: single OT-induced PRL surge induces delayed DA surge. *D*: OT is elevated transiently due to simulated OT injection, but returns to basal levels within 1 day of injection.

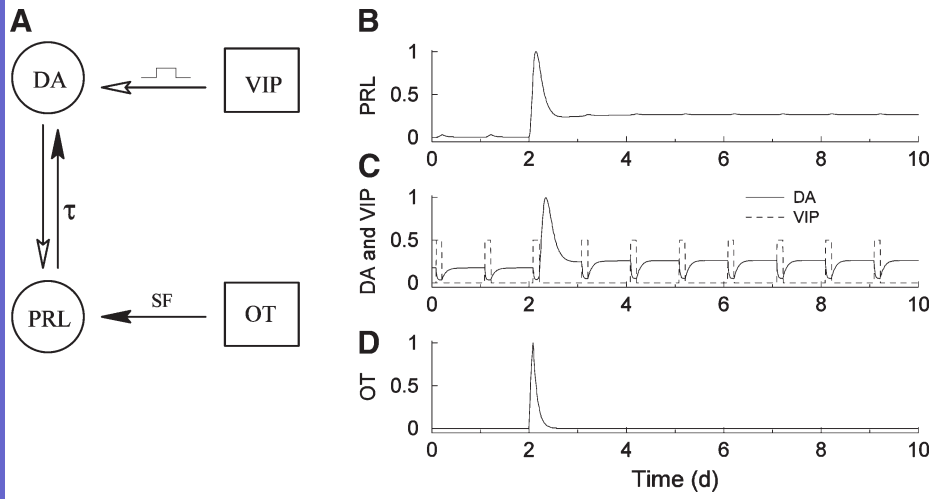

Fig. 5. *A*: OT stimulates lactotrophs, activating long-lasting stimulatory factor (SF). *B*: OT injection results in an initial PRL surge, with sustained level of activation due to SF. However, circadian PRL rhythm is not initiated. *C*: SF increases activity level of DA due to its effect on PRL. *D*: as in previous scenario, OT levels return to baseline within 1 day after injection.

sider the possibility that a population of hypothalamic OT-sensing neurons exists and that these neurons are inhibitory to DA neurons. To ensure that OT has a long-lasting effect, we assume that the OT-sensing neurons have "off" and "on" stable states, and may be in either state at any time. These neurons are thus "bistable," because the two stable states coexist, and the neurons can be perturbed from one stable state to another by brief perturbations, such as OT injection.

To demonstrate one realization of these OT-sensing bistable neurons, we introduce a variable, N, that represents the activity level of the neurons. The first term in the differential equation for N reflects the stimulatory effect of OT, v_{on}OT. The second term encodes the bistability of the neurons, $r_nN(N - 1/2)(N - 1)$. This cubic function has three roots, $N_1^* = 0$, $N_2^* = 1/2$, and $N_3^* = 1$. The first and third roots (N_1^* and N_3^*) correspond to steady states that are both stable; $N_1^* = 0$ corresponds to the "off" state and $N_3^* = 1$ to the "on" state (Fig 6). The middle root (N_2^*) is unstable and is the threshold between the two stable states. The differential equation for the activity level of OT-sensing neurons is then

$$\frac{dN}{dt} = v_{on}\text{OT} - r_nN(N - 1/2)(N - 1) \qquad (8)$$

We assume that the OT-sensing neurons have an inhibitory action on DA neurons (Fig. 7*A*), so that when N is in an "on" state ($N = 1$), the tonic stimulatory drive to DA neurons is removed, reducing the activity level of the neurons. The DA differential equation, *Eq. 4*, becomes

$$\frac{d\text{DA}}{dt} = T_d(1 - N) + T_dk_p\text{PRL}_\tau^2 - q\text{DA} - r_v\text{VIP}\cdot\text{DA} \qquad (9)$$

The PRL differential equation is given by *Eq. 5*, where no SF is present; that is, OT has only a transient direct effect on the lactotrophs.

Figure 7 demonstrates that OT injection can trigger a sustained PRL rhythm through the action of OT-sensing neurons that are inhibitory to DA neurons. Before OT injection, N is near 0 (the "off" state; Fig. 7*E*) and the DA neurons are uninhibited, so DA is elevated (Fig. 7*C*). This keeps PRL at a low level (Fig. 7*B*). The OT injection (Fig. 7*D*) perturbs the OT-sensing neurons from the "off" state N_1^* up above the threshold N_2^* to the "on" state $N_3^* = 1$. N stays at this upper stable state unless perturbed back down to the "off" state. With N elevated (Fig. 7*E*), the DA neurons receive tonic inhibition, reducing the DA activity level so that the PRL rhythm can emerge. This rhythm is identical to that shown in Fig. 3, with DA oscillating out of phase with PRL, because the governing equations are identical.

We thus predict that OT injection initiates and maintains the PRL rhythm by indirectly inhibiting the DA neurons, rather than directly stimulating the lactotrophs. We suggest a scenario in which OT injection triggers the PRL rhythm, OT-sensing neurons act as a bistable switch or memory device to maintain the rhythm, and PRL-DA interactions generate the rhythm. Once triggered, the circadian rhythm persists indefinitely unless suppressed by some other factor, which is the focus of the next section.

Two potential targets for the rhythm-terminating signal. The PRL rhythm is typically terminated abruptly, 10 days after its initiation in pregnant rats (10) or 12 days after initiation if the mating is sterile or copulomimetic (23). In pregnant rats, the terminating factor appears to be the hormone PL-1 (54), which reaches a high concentration in the blood by *days 9–10* of pregnancy (52). This is associated with high levels of DA neuron activity, which suggests that PL-1 acts by stimulating DA neurons (14). By contrast, in ovariecto-

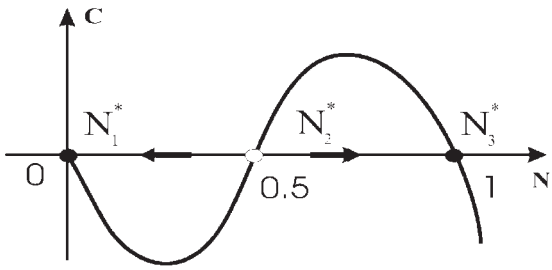

Fig. 6. Bistability of OT-sensing neurons. Axes are N and cubic term in *Eq. 7*, $C(N) = -r_nN(N - 1/2)(N - 1)$. Intersections with N-axis are steady states. Arrows indicate direction in which N will move if started to left or right of $N_2^* = 0.5$.

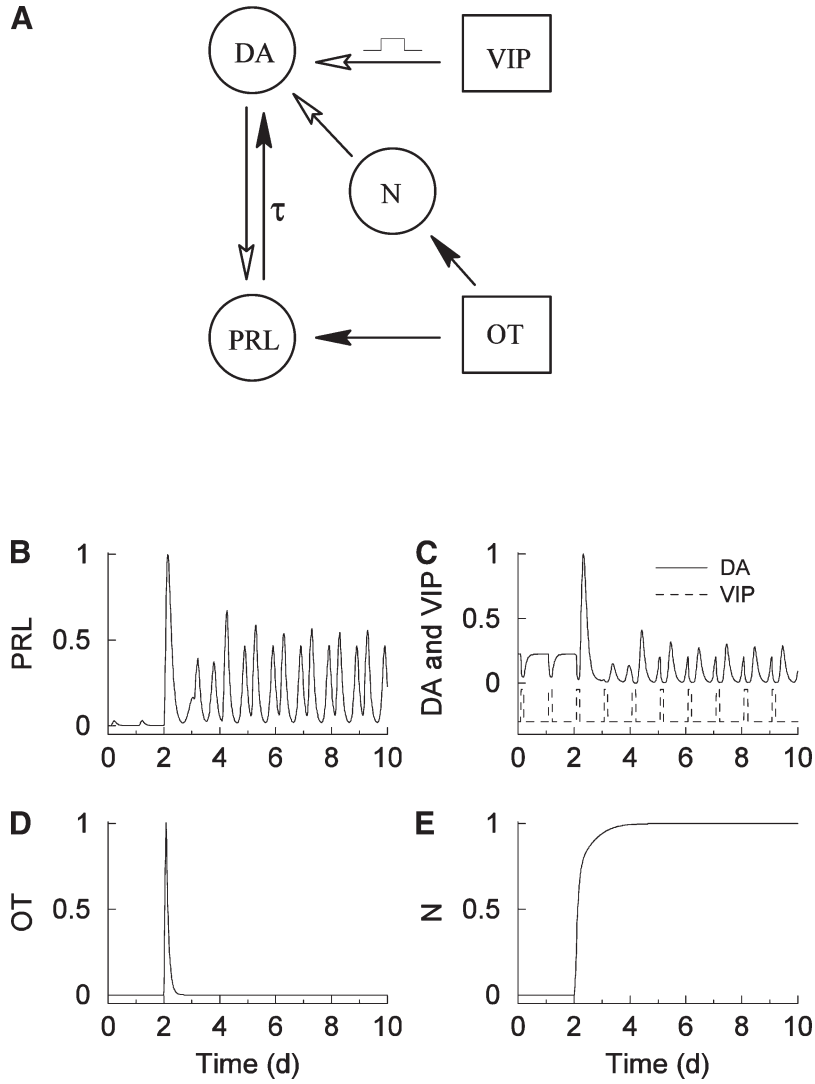

Fig. 7. *A*: population of bistable OT-sensing neurons (*N*) is added to network model. These are activated by OT and are inhibitory to DA neurons. *B*: activation of OT-sensing neurons initiates and maintains circadian PRL rhythm. *C*: overall DA activity level is reduced by OT-sensing neurons, but DA activity oscillates out of phase with PRL, as in Fig. 3. *D*: OT level returns to baseline within 1 day after OT injection. *E*: OT-sensing *N* are perturbed from "off" state ($N = 0$) to "on" state ($N = 1$) by transient OT injection.

mized CS rats, the identity of the terminating factor is not known.

We investigated two potential mechanisms for the termination of the PRL rhythm in ovariectomized rats. In the first model, the terminating factor directly inhibits lactotrophs. In the second model, the terminating factor stimulates DA neurons. Our goal is to determine the effectiveness of each mechanism for rhythm termination and to identify characteristics that distinguish the two.

The terminating factor (denoted as *H*) is simulated the same way in both models. Initially, $H = 0$, and *H* increases to 1, with a time constant of 40 h starting 11 days after induction of the PRL rhythm (Fig. 8*E*). This process is described by the following differential equation:

$$\frac{dH}{dt} = \mathrm{SW} \times (1 - H)/\tau_H, \qquad (10)$$

where SW is a switching or Heaviside function that is 0 for the first 11 days after rhythm induction, and 1 thereafter. The parameter τ_H is the time constant for the rise in *H* to its new equilibrium value of 1.

In the first model, *H* acts directly on the lactotrophs as an inhibitory factor, so the PRL equation becomes

$$\frac{d\mathrm{PRL}}{dt} = \frac{T_p}{1 + k_d \mathrm{DA}^2} - q\mathrm{PRL} + v_o\mathrm{OT} - r_H\mathrm{PRL}\cdot H, \qquad (11)$$

where the *H* inhibition is introduced in a multiplicative manner, as was the VIP inhibition of DA neurons in *Eq. 4*. A simulation of the full sequence of events with this model, from OT-induced triggering to *H*-induced termination, is shown in Fig. 8. The rise in *H* that begins on *day 13* (11 days after rhythm initiation by OT) successfully terminates the rhythm. The decline in PRL results in a decline in DA activity; so with this mechanism the DA level is lower after termination of the rhythm than before or during the rhythm. Thus termination of the rhythm by this mechanism is inconsistent with rhythm termination in pregnant rats, where the DA level is elevated throughout the second half of pregnancy (1). Although it has not been shown, it is likely that the DA level is also elevated after pseudopregnancy, because the estrus cycle begins immediately at this time.

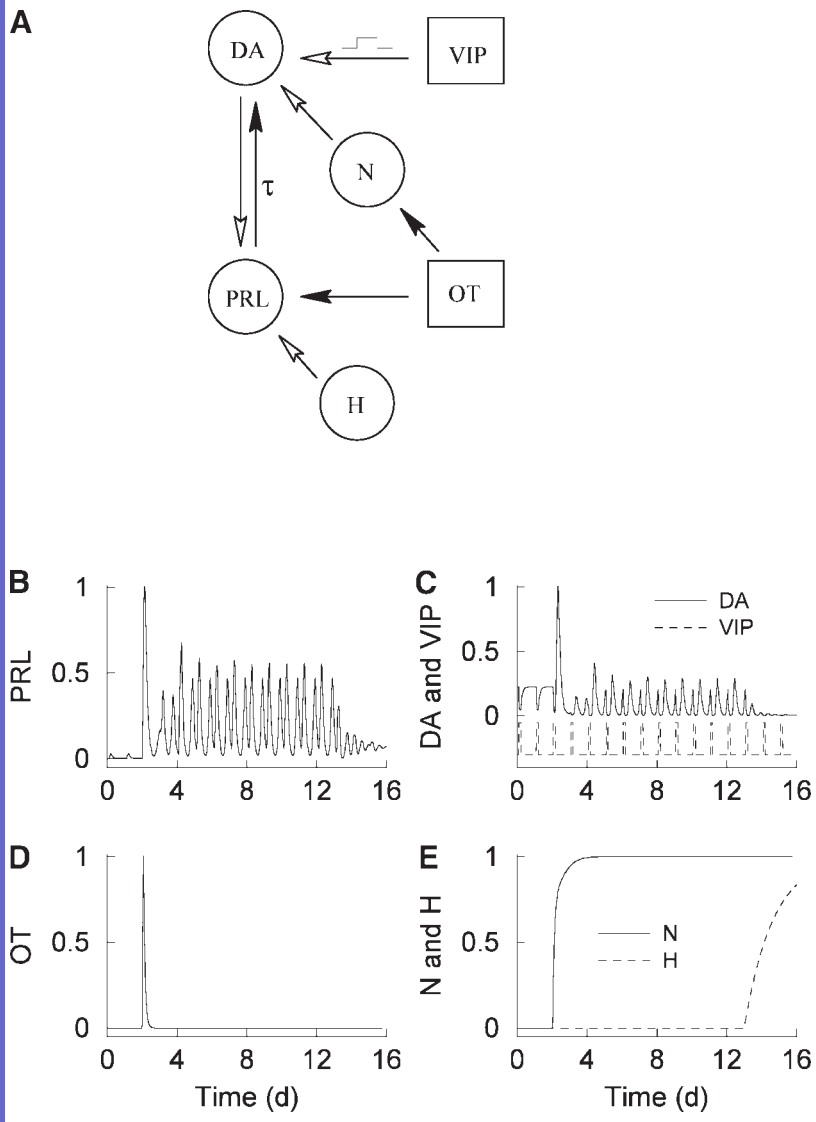

Fig. 8. *A*: model in which terminating factor (*H*) directly inhibits lactotrophs. *B*: PRL rhythm is terminated ~12 days after induction. *C*: final DA level is lower than before or during PRL rhythm. *D*: OT injection triggers rhythm but plays no other role. *E*: OT-sensing neurons (*N*) maintain rhythm, whereas factor *H* terminates rhythm. *Equation 11* is used, with $r_H = 5$.

In the second model, *H* acts indirectly on lactotrophs by stimulating DA neurons. The PRL equation is as it was in *Eq. 5*, whereas the factor $r_H H$ is added to the DA differential equation

$$\frac{d\mathrm{DA}}{dt} = T_d(1 - N) + T_d k_p \mathrm{PRL}_\tau^2 - q\mathrm{DA} - r_v \mathrm{VIP}\cdot\mathrm{DA} + r_H H \quad (12)$$

A simulation of the full sequence of events with this model is shown in Fig. 9. As with the previous termination mechanism, the PRL rhythm is effectively stopped once *H* rises to its new equilibrium value. Unlike the previous model, the DA level is now elevated upon rhythm termination. Thus this mechanism is consistent with rhythm termination in pregnant rats, where the termination factor, PL-1, is in fact known to stimulate DA neurons.

We note that a related mechanism for rhythm termination is to reset *N* to the "off" state, thereby removing inhibition of DA neurons. This would have the same effect as stimulating DA neurons and would have the additional benefit of resetting the population of OT-sensing neurons to their original state.

In summary, model simulations demonstrate that either direct inhibition of lactotrophs or stimulation of DA neurons can terminate the PRL rhythm. They also predict that the DA level will be low after rhythm termination if the termination mechanism is direct, sustained inhibition of lactotrophs. The DA level will be high after rhythm termination if the mechanism is stimulation of DA neurons.

DISCUSSION

We have developed a mathematical model for the initiation, maintenance, and termination of the circadian PRL rhythm induced by OT injection in female rats. This rhythm is similar to that induced by the mating stimulus. The model leads to several predictions. First, the model predicts that the mechanism for the rhythm is the mutual interaction between lactotrophs and hypothalamic DA neurons. The time delay for the stimulatory action of PRL on the DA neurons is the crucial parameter that allows the rhythm to occur and sets the free-running oscillation period. Second, the model predicts that one or more factors in addition to VIP contribute to the unequal

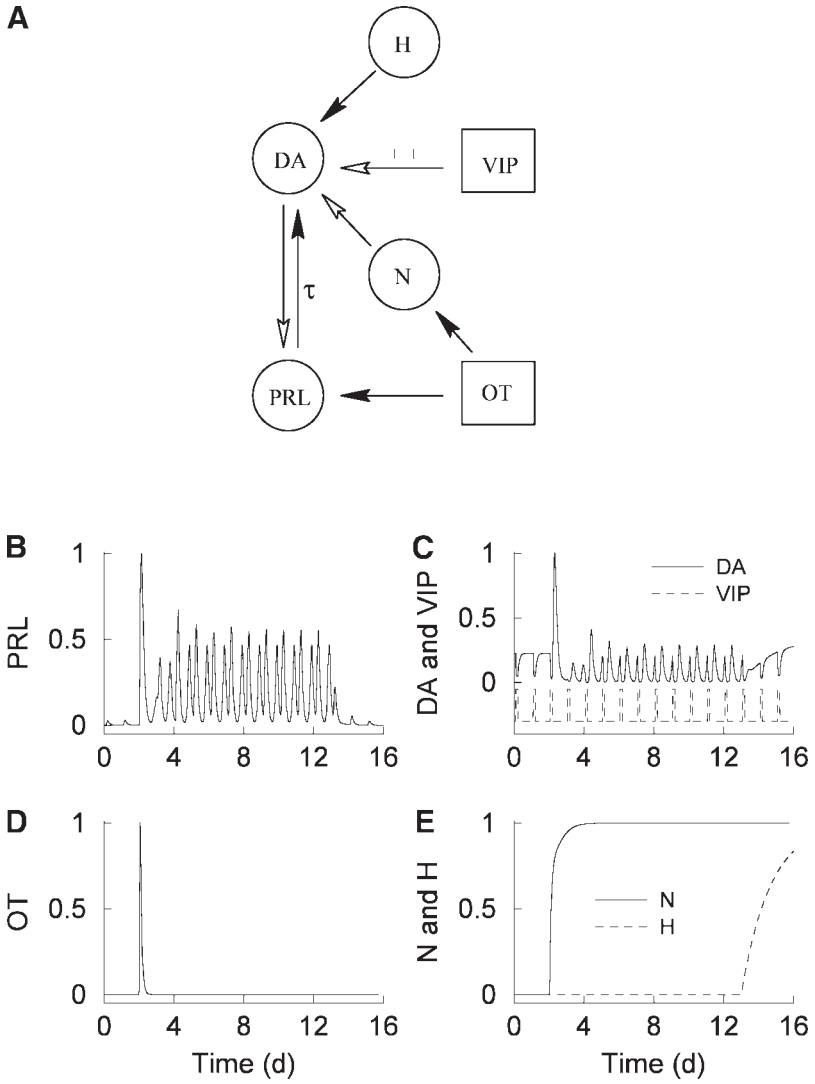

Fig. 9. *A*: rhythm-terminating hormone *H* stimulates DA neurons in this model. *B*: PRL rhythm is successfully terminated ~12 days after induction. *C*: DA level is higher after rhythm termination than before or during rhythm. *D*: OT injection triggers rhythm, but plays no other role. *E*: OT-sensing neurons *N* maintain rhythm, whereas factor *H* terminates rhythm. *Equation 12* is used, with $r_H = 15$.

daily PRL pulse amplitudes. In the model, if VIP input is removed, the PRL rhythm continues but with equal pulse amplitudes. However, it has been shown that the pulse amplitudes are unequal even in constant darkness (6, 55), a condition in which rhythms in VIP mRNA (49) and protein content (45) are abolished. One factor that may contribute to the larger morning PRL surge is OTergic input from the PVN, which is stimulatory to lactotrophs (17), but there are other potential factors.

The model also predicts that, although OT injection triggers the PRL rhythm, it is not responsible for its maintenance. We describe a putative "memory" mechanism that consists of a population of bistable OT-sensing neurons. Bistable neurons have been described before in other contexts (9). In our model, the bistable neurons are postulated to innervate the DA neurons and are switched from an "off" to an "on" state by OT injection. We further suggest that this mechanism may apply to the mating-induced rhythm, where OT is released in response to the mating stimulus. Finally, the model illustrates two potential mechanisms for termination of the PRL rhythm, leading to two distinct outcomes in DA activity. If rhythm termination is through direct sustained inhibition of lactotrophs, then the DA activity level will be lower, once the rhythm has been terminated, than during or before the rhythm. If rhythm termination is through stimulation of DA neurons, then the DA activity level will be higher upon rhythm termination than during the rhythm. The latter mechanism seems more plausible, both in pregnant and in pseudopregnant rats.

There is considerable experimental evidence for the proposed oscillatory mechanism. Mutual feedback between the two cell populations is well established (4, 22). Also, there is evidence for a time delay of PRL stimulatory action on DA neurons (15), although additional studies are needed to better quantify this delay. Finally, we demonstrated that an essential feature of the PRL-DA oscillation mechanism is that peaks in PRL secretion and DA neuron activity are out of phase with one another. This out-of-phase relationship has been demonstrated in pregnant rats (35, 40), OVX CS rats (38), and OT-injected ovariectomized rats (18). An earlier model for the PRL rhythm predicted that DA levels would be low in the morning and higher during the remainder of the day (17), a time course that is at odds with the experimental data.

The postulated entraining action of VIP neurons of SCN origin is supported by several experimental studies. It has been

shown (25) that DA neurons of the arcuate nucleus express VIP receptors, and that VIP fibers originating in the SCN innervate DA neurons of the arcuate nucleus. Furthermore, it has been shown (26) that VIP inhibits DA neuron activity. Finally, it has been shown (26, 43, 45) that, under normal lighting conditions, VIP neurons of the SCN have elevated activity in the early morning, which can serve as an entraining signal to the PRL-DA circuit.

Evidence that OT triggers the PRL rhythm is reported in the companion paper (18). We considered the possibility that OT acts directly on lactotrophs to initiate the rhythm and found that this mechanism is not effective (Figs. 4–5). Because evidence for OT receptors on neuroendocrine DA neurons is lacking, we postulated the existence of a population of OT-sensing neurons that innervate the DA neurons. The identity of these neurons is unknown, and thus the existence of this neural population serves as a model prediction that we intend to investigate in the future.

Each differential equation in our model contains several parameters, and the values of these parameters have been chosen to produce the circadian PRL rhythm that is observed experimentally. We have performed a sensitivity analysis and found that the PRL rhythm is primarily sensitive to changes in the time delay τ. The effect of changes in this parameter on the period of the rhythm is shown in Fig. 2. The rhythm is more robust to changes in other parameters.

Our model is minimal in the sense that many factors that could affect rhythmic PRL secretion are not included (22). Some of these factors could be incorporated into future models. It has been demonstrated (17, 20, 34) that OT is a PRL-releasing factor, and it is possible that OT neurons in the paraventricular nucleus (PVN) affect that PRL rhythm. Indeed, we (17) have shown that these neurons display rhythmic activity during the PRL rhythm. These OT neurons have not been included in the minimal model because many aspects of their interaction with lactotrophs and the SCN are unclear. For example, although we have shown that OT neurons of the PVN express VIP and PRL receptors (17), we do not yet know whether VIP and PRL stimulate or inhibit OT neurons. Serotonin may also play a role in the PRL rhythm. Although serotonin does not stimulate PRL release in vitro (33), it may function as a neurotransmitter acting on hypothalamic DA or OT neurons. Other factors that affect PRL secretion and thus may affect the PRL rhythm, include thyrotropin-releasing hormone (7, 50), among others (22). PRL itself binds to PRL autoreceptors on lactotrophs and inhibits PRL release (5), so it may play some role in the PRL rhythm. Although these many factors may play roles in the PRL rhythm, our simulations demonstrate that the rhythm can be triggered, maintained, and terminated using a model with just a few key components.

ACKNOWLEDGMENTS

We thank Joel Tabak for a critical reading of the manuscript.

GRANTS

This work was supported by National Institute of Drug Abuse Grant no. DA-19356 to R. Bertram, M. Egli, and M. E. Freeman, and National Institute of Diabetes and Digestive and Kidney Diseases Grant no. DK-43200 to M. E. Freeman.

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