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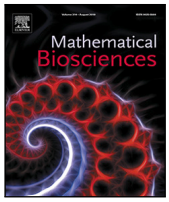

Original research article

A mathematical model of melatonin synthesis and interactions with the circadian clock

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ABSTRACT

A new mathematical model of melatonin synthesis in pineal cells is created and connected to a slightly modified previously created model of the circadian clock in the suprachiasmatic nucleus (SCN). The SCN influences the production of melatonin by upregulating two key enzymes in the pineal. The melatonin produced enters the blood and the cerebrospinal fluid and thus the SCN, influencing the circadian clock. We show that the model of melatonin synthesis corresponds well with extant experimental data and responds similarly to clinical experiments on bright light in the middle of the night. Melatonin is widely used to treat jet lag and sleep disorders. We show how the feedback from the pineal to the SCN causes phase resetting of the circadian clock. Melatonin doses early in the evening advance the clock and doses late at night delay the clock with a dead zone in between where the phase of the clock does not change.

1. Introduction

Circadian rhythms play an important role in human health and disease. Many physiological quantities such as blood pressure, heart rate, venous CO_2, body temperature, metabolic rate, enzyme activities and even endocrine and immune system variables have a 24-hour rhythm [1,2]. There is manifold evidence that disturbances in circadian timing interfere with the sleep-wake cycle and underlie certain sleep-wake disorders [3,4]. In addition, some affective disorders, such as bipolar disorder and depression, are associated with disrupted circadian rhythms [5].

The identification of the suprachiasmatic nucleus (SCN), in the hypothalamus, as the master circadian regulator is credited to R. Moore [6], who traced the light input to the eyes through the retino-hypothalamic tract to the SCN. The molecular and genetic basis of the circadian clock is attributed to J. Hall, M. Rosbash, and M. Young, whose transcription–translation feedback loop model earned a Nobel prize in 2017 [7]. Their work is the basis for our simple SCN mathematical model.

In mammals, many cells in the central nervous system and periphery have circadian clocks; these cellular clocks are synchronized hierarchically, with the synchronized cells of the SCN acting as the master clock. An interesting and important question is how the SCN communicates timing to other parts of the brain and other organs and glands in the body [8]. For other central clocks and the pineal and adrenal glands, direct and indirect neural projections convey this timing information [9,10], resulting in hormonal signals to peripheral organs. The primary neural projection from the SCN is to the paraventricular nucleus (PVN), which projects to the parasympathetic region of the brainstem and sympathetic neurons in the spinal cord [11]. A sympathetic noradrenergic projection from the PVN to the pineal gland stimulates enzymes in the pineal that produce melatonin [11,12]. Melatonin is released from the pineal into both the blood and the cerebrospinal fluid (CSF), and thus becomes a whole body messenger of the current state of the clock [13]. Interestingly, melatonin itself affects the SCN [13] and is involved in phase resetting of the master clock.

We have made a mathematical model of the synthesis of melatonin in pineal cells, the stimulation of the pineal by the PVN, the release of melatonin into the blood and the CSF where it influences the clock in the SCN (see Fig. 1). There have been a large number of mathematical models addressing clock mechanisms [14,15], synchronization of cells that have clock mechanisms [16,17], temperature compensation [18–23] and light inputs [24]. There have also been other models of melatonin release and phase resetting [25–32]. None of these other models have a mechanistic model of the circadian clock and only one [30] includes the molecular synthesis of melatonin.

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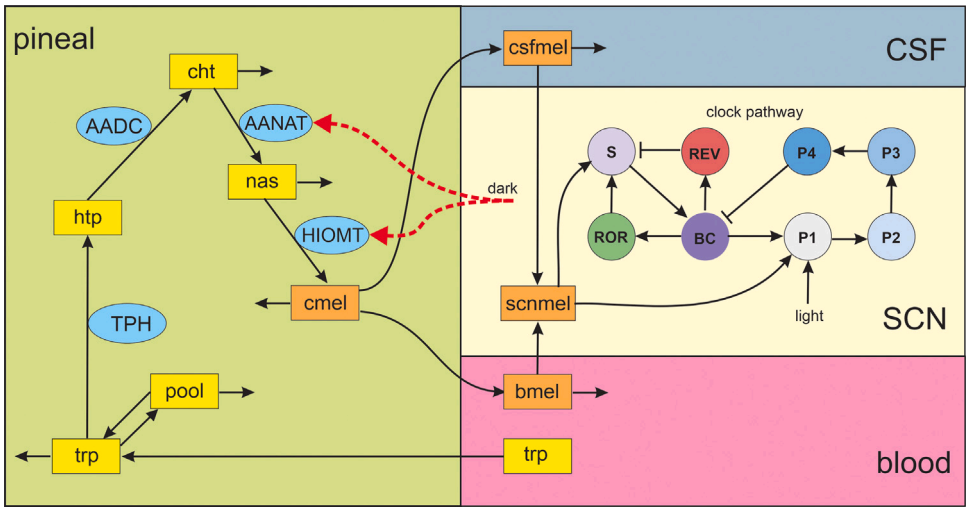

Fig. 1. The model of melatonin synthesis and the SCN. For the melatonin synthesis model the substrate acronyms are in rectangular boxes. Full names are given in Table 1. The enzymes in the blue ovals are: TPH, tryptophan hydroxylase; AADC, aromatic amino acid decarboxylase; AANAT, serotonin N-Acetyltransferase; HIOMT, hydroxyindole-O-methyltransferase. In the SCN model, the variables are in circles and the full names are given in Table 3. The SCN affects the pineal, since, at night a series of neural projections releases norepinephrine in the extracellular space around pineal cells and activates the enzymes AANAT and HIOMT. And the pineal releases melatonin into the blood and the cerebrospinal fluid and the resulting increase in melatonin in the SCN activates Per1.

In Section 3.1, we show that the concentrations in our melatonin synthesis model, including blood melatonin, correspond well to the experimental measurements of Klein [12,33–35] during day and night. In Section 3.2, we use the model to study how the concentrations of melatonin in the blood (*bmel*) and the cerebrospinal fluid (*csfmel*) depend on the expression level of AANAT at night. As AANAT expression level increases, both *bmel* and *csfmel* increase but with a much smaller slope when AANAT expression is high, because an increase in AANAT expression is partly compensated by a decrease of serotonin. In Section 3.3 we study how concentrations change if bright light is turned on in the middle of the night and we compare the model predictions to Czeisler's clinical experiments [36]. Finally, in Section 3.4 we study how the dosing of melatonin can cause phase shifts in the circadian clock and compare to experimental data.

2. Methods

We have created a new mathematical model for the production of melatonin in pineal cells; it is described in the first subsection and depicted schematically in Fig. 1. We also use a slightly modified previous model of the circadian clock in the SCN [37–39]; It is described in the second subsection. The clock in the SCN influences the production of melatonin by changing the activity levels of the enzymes AANAT and HIOMT (red dashed arrows in Fig. 1). And, the melatonin produced influences the clock via the concentrations of melatonin in the plasma and the CSF.

2.1. The melatonin synthesis model

The mathematical model for melatonin consists of 8 differential equations for the variables whose full names are listed in Table 1. See the diagram in Fig. 1.
The differential equations are:

$$\frac{d[trp]}{dt} = V_{trpin} - V_{\mathrm{TPH}}(trp) - V_{\mathrm{pool}}(trp, pool) - k_{trp}^{catab} \cdot trp. \quad (1)$$

$$\frac{d[pool]}{dt} = V_{\mathrm{pool}}(trp, pool) - k_{pool}^{catab} * pool. \quad (2)$$

$$\frac{d[htp]}{dt} = V_{\mathrm{TPH}}(trp) - V_{\mathrm{AADC}}(htp). \quad (3)$$

$$\frac{d[cht]}{dt} = V_{\mathrm{AADC}}(htp) - V_{\mathrm{HTcatab}}(cht) - AT(t) \cdot V_{\mathrm{AANAT}}(cht). \quad (4)$$

$$\frac{d[nas]}{dt} = AT(t) \cdot V_{\mathrm{AANAT}}\ (cht) - HO(t) \cdot V_{\mathrm{HIOMT}}(nas) - k_{nas}^{catab} \cdot nas. \quad (5)$$

$$\frac{d[cmel]}{dt} = HO(t) \cdot V_{\mathrm{HIOMT}}(nas) - 2.2. * cmel + (15000) * bmel \quad (6)$$

$$-(0.01) * (2.2 * cmel + 500. * csfmel) - k_{cmel}^{catab} * cmel. \quad (7)$$

$$\frac{d[bmel]}{dt} = (2.2/15000) * cmel - 1 * (bmel) - k_{bmel}^{catab} * bmel. \quad (8)$$

$$\frac{d[csfmel]}{dt} = (0.01) * (2.2/500 * cmel - 1 * csfmel) - k_{csfmel}^{catab} * csfmel. \quad (9)$$

Table 1
Names of variables.

In equations and text	Full name
trp	Cytosolic tryptophan
pool	Cytosolic tryptophan pool
htp	5-hydroxytryptamine
cht	Cytosolic serotonin
nas	N-acetyl-serotonin
cmel	Cytosolic melatonin
bmel	Plasma melatonin
csfmel	CSF melatonin

The melatonin synthesis pathway is indicated in the green part of Fig. 1. The acronyms of the substrates are in the rectangular boxes and the acronyms of the enzymes that catalyze the reactions are in the blue ovals. Most reactions are Michaelis–Menten and the V_{max} values and K_m values that we use are given in Table 2. We give references for the K_m values. V_{max} values in the literature are notoriously variable and context dependent, so we choose them so that the substrates are within known ranges. In the following, we walk through the melatonin synthesis pathway discussing the major modeling issues.

In pineal cells, melatonin is made from serotonin (cht), which, in turn, is made from tryptophan (trp) imported from the blood. We assume that trp in the blood is constant at 96 μM [40]. The V_{max} of the transporter that brings trp in from the blood to pineal cells is much higher than in our previous models of serotonin varicosities. This is because serotonin is approximately 500 μM during the day in pineal cells [33], while serotonin is much lower in serotonin varicosities [41]. Trp is imported into all cells and used in protein synthesis and *pool* represents the trp used for protein synthesis and other uses.

As described in the introduction, the key enzymes that control melatonin synthesis are AANAT and HIOMT that are activated during darkness by norepinephrine projections from the SCN (the red dashed

Table 2
Kinetic parameters (μM, μM/h, /h).

Velocity	Parameter	Model value	Literature value	References
V_{trpin}	Neutral amino acid transporter			
	K_m	330	64	[40]
	V_{max}	9000		[a]
V_{POOL}	Linear exchange between *trp* and *pool*			
	k_{topool}	9		[a]
	$k_{frompool}$	0.6		[a]
V_{TPH}	Tryptophan hydroxylase			
	K_{trp}	40	40	[46]
	V_{max}	695		[a]
	K_i (substrate inhibition)	1000	970	[46]
V_{AADC}	Aromatic amino acid decarboxylase			
	K_m	160	160	[47]
	V_{max}	900		[a]
V_{HTcatab}	Catabolism of serotonin			
	K_m	95	94–95	[48,49]
	V_{max}	568		[a]
V_{AANAT}	Arylalkylamine-N-acetyltransferase			
	K_m	1235	338–1235	[43,50]
	V_{max} (day)	62.5		[a]
	V_{max} (night)	29*(62.5)		[a]
V_{HIOMT}	Hydroxyindole-O-methyltransferase			
	K_m	40	7.7–100	[43,51]
	V_{max} (day)	336		[a]
	V_{max} (night)	(1.3)*336		[a]
Catabolism				
	k_{trp}^{catab}	1		[a]
	k_{pool}^{catab}	2		[a]
	k_{ca5ht}^{catab}	1		[a]
	k_{cmel}^{catab}	2.3		[a]
	k_{bmel}^{catab}	7		[a]
	k_{csfmel}^{catab}	7		[a]

[a] See text.

arrows in Fig. 1). In our model, the clock starts at 12 noon and "dark" is from 8 pm until 6 am. We do not model the pathway from the SCN to the pineal explicitly, but, instead, multiply the V_{max} of V_{AANAT} and V_{HIOMT} by the following functions, respectively:

$$AT(t) = \begin{cases} 1 & \text{if } 0 \le t < 8, \\ 1 + (28)\frac{(t-8)^2}{15+(t-8)^2} & \text{if } 8 \le t \le 18, \\ 1 + (24)e^{-10(t-18)} & \text{if } 18 < t. \end{cases}$$

$$HO(t) = \begin{cases} 1 & \text{if } 0 \le t < 8, \\ 1 + (.3)\frac{(t-8)}{2+(t-8)} & \text{if } 8 \le t \le 18, \\ 1 & \text{if } 18 < t. \end{cases}$$

These two functions can be seen in the differential equations above. Therefore, in the model the activity of $AANAT$ can increase by a maximal factor of 29 and the activity of $HIOMT$ can increase by a maximal factor of 0.3, corresponding to the ranges of values indicated in the literature [12,33,42,43].

Finally, cytosolic melatonin in pineal cells diffuses into the plasma and into the CSF as indicated explicitly in the 6th, 7th, and 8th differential equations (labeled (6), (8), (9)). The extra large factors in these equations reflect the differences in volumes of the pineal (0.2 ml) [44], the plasma (3000 ml), and the CSF (100 ml) [45].

2.2. The molecular clock model

The circadian clock model used in this paper is adapted from previous work [37–39]. BMAL1-CLOCK (BC) activates the transcription of PER. The variables P_1 to P_4 represent PER proteins that are progressively phosphorylated. S represents a precursor to BC, which can be thought of as Bmal1 mRNA. Orphan nuclear receptors REV-ERBs (REV) inhibit S and retinoic acid-related orphan receptors (RORs) activate S. All the variables in the molecular clock model are listed in Table 3.

Table 3
Names of variables.

Variables	Full name
P_i	PERIOD (PER) proteins
BC	BMAL1-CLOCK heterodimer
S	Bmal1 mRNA
REV	Orphan nuclear receptors REV-ERBs
ROR	Retinoic acid-related orphan receptors (RORs)
M_{csf}	Normalized CSF melatonin
M_{dose}	Melatonin dose

The production term for P_1 depends on $f(BC, P_4)$, the concentration of available BC not bound to the inhibitor P_4; see Eqs. (10) and (14). $f(BC, P_4)$ is a simplification of the protein sequestration model used in [52–54] and derived in [55,56]. We assume tight binding of P_4 to BC.

$$f(BC, P_4) = \frac{BC - P_4 + |BC - P_4|}{2} = \begin{cases} BC - P_4 & BC > P_4 \\ 0 & BC \le P_4. \end{cases} \tag{10}$$

Light stimulates the expression of Per genes in the SCN [57–59]. The mathematical model with light input is in one of two states (light or dark) depending on time.

$$L(t) = \begin{cases} 1.3 & \text{if } 0 \le \mathrm{mod}(t, 24) < 8 \\ 0.7 & \text{if } 8 \le \mathrm{mod}(t, 24) < 18 \\ 1.3 & \text{if } 18 < \mathrm{mod}(t, 24). \end{cases} \tag{11}$$

In Section 3.4 we study the phase-shifting effects of melatonin administration on the circadian clock. $M_{csf}(t)$ denotes the melatonin signal from *csfmel*, normalized to the mean concentration. Melatonin administered at subjective dusk is known to stimulate Per expression [60]. P_1 in the model is periodically forced by both the melatonin signal and a light signal; see Eq. (14). It is also known that melatonin stimulates ROR activity, contributing to the upregulation of Bmal1 [61]. This effect is captured in Eq. (19). In Section 3.4 we will

see that the influence of melatonin on Per transcription (Eq. (14)) alone is strong enough to explain the phase-shifting response of the circadian clock to melatonin administration.

The complete set of differential equations for the circadian clock with melatonin input is given by

$$M_{total}(t) = M_{scn}(t) + M_{dose}(t) \tag{12}$$

$$\frac{dM_{dose}}{dt} = -\frac{3\ln 2}{2} M_{dose} \tag{13}$$

$$\frac{dP_1}{dt} = M_{total}(t) + r_1 L(t) f(BC, P_4) - r_2 P_1 \tag{14}$$

$$\frac{dP_2}{dt} = r_2 P_1 - r_3 P_2 \tag{15}$$

$$\frac{dP_3}{dt} = r_3 P_2 - r_4 P_3 \tag{16}$$

$$\frac{dP_4}{dt} = r_4 P_3 - d_4 P_4 \tag{17}$$

$$\frac{dBC}{dt} = \beta_{bc} S - d_{bc} BC \tag{18}$$

$$\frac{dS}{dt} = \beta + \alpha f(S, REV) \cdot \frac{ROR(1 + M_{total}(t))}{2} - d_s S \tag{19}$$

$$\frac{dREV}{dt} = r_{rev} f(BC, P_4) - d_{rev} REV \tag{20}$$

$$\frac{dROR}{dt} = r_{ror} f(BC, P_4) - d_{ror} ROR. \tag{21}$$

In the model, we administer melatonin doses through the function $M_{dose}(t)$. Administered melatonin M_{dose} is taken to have a half-life of 40 min [13]. M_{scn} is simplistically taken to be a normalized average of *bmel* and *csfmel*, $M_{scn} = \frac{M - mean(M)}{max(M) - min(M)}$, where M is the average of *bmel* and *csfmel*. We measure the phase-shifting effects of melatonin doses on the SCN molecular clock by nonlinear least-squares fitting functions of the form $g(t) = a\cos(bt - c) + d$ to $P_2(t)$ with and without the melatonin dose for a period of 24 h after administration. Then, we use the finddelay function in MATLAB to approximate their phase difference during the 24 h after melatonin administration, where positive values indicate a phase advance and negative values indicate a phase delay.

3. Results

3.1. Synthesis of melatonin

In the 1970's, Klein published what has become an iconic figure describing the synthesis of melatonin and how enzyme activities and the levels of melatonin and its precursors depend on day and night [12,33–35]. During darkness, neurons in the SCN stimulate the paraventricular nucleus (PVN), which results in the firing of cells in the superior cervical ganglia (SCG) that project to the pineal gland where they release norepinephrine in the extracellular space. The result is a very large increase in activity of the enzyme AANAT (20–30 fold) and a modest increase in the enzyme HIOMT (30%). These increases in enzyme activities cause the pineal cells to produce large amounts of melatonin via the pathway in Fig. 1 during darkness. As described in Methods, we do not model the details of the pathway from the SCN to the pineal, but, instead, simply assume the upregulation of AANAT and HIOMT during darkness. The results can be seen in Fig. 2 that shows the cellular, blood and cerebrospinal fluid concentrations throughout day and night. We start our day at noon (t = 0) and darkness is from 8 pm (t = 8) until 6 am (t = 18).

The shapes of the curves in Fig. 2 are quite similar to the Klein data displayed in [12,33–35], however their y-axes are in log units and we report velocities in Panels B and D while they report "enzyme activity" measured at a fixed concentration of their respective substrates. Pineal cells have high concentrations of serotonin during the day (500 μM as compared to 2 μM in the cytosol of serotonin varicosities [41]) and are quite depleted at night (panel A). The velocity of the AANAT reaction rises quickly after dark begins and drops quickly in the morning (Panel B). The concentration of *nas* follows a similar pattern (Panel C) but does not rise as much because the concentration of *cht* falls during the night. Even though the activity of HIOMT only rises by 30% at night, the velocity of the HIOMT reaction follows the same pattern (Panel D) because it is driven by the rise in *nas*. Cytosolic melatonin (Panel E) and blood and CSF melatonin (Panel F) show the same pattern, though note that *bmel* and *csfmel* are in picomolar. The sharp rise, relatively flat concentrations during night, and sharp fall in the morning are caused by our assumptions about the rapid rise and decay of AANAT activity. Some animals show slower rise and more rounded profiles [35]; these can be obtained by changing the rise and fall rates of AANAT activity. Overall, the day and night values of the concentrations in our model are similar to the values in the experimental literature; see Table 4.

Table 4
Day-night values of variables.

	Model(day)	Model(night)	Exper(day)	Exper(night)	Reference
cht (μM)	506	140	500	150	[33]
nas (μM)	3.6	49	3	40	[33]
cmel (μM)	3.5	26.6	2	30	[33]
bmel (pM)	64.3	496	20–70	350–550	[62]
csfmel (pM)	18.7	173	8–20	120–180	[63–65]

The wide ranges of blood melatonin and CSF melatonin in the literature are because measurements are made on different mammals and there are large species and individual differences [66]. We give the day and night values for the fixed model used for Figs. 2, 3, and 4. By varying parameters, we would obtain ranges instead of specific values.

3.2. Dependence on the activity of AANAT

Since melatonin is synthesized from serotonin, a large number of genetic studies have found associations between gene polymorphisms in AANAT and affective disorders, dysregulation of the sleep-wake cycle, and other disorders. For example, Pagan et al. [67] reports on the relationship between AANAT activity and blood melatonin in normal and autism spectrum patients.

Kripke et al. [68] report on clear relations between AANAT polymorphisms and sleep disorders but a more nuanced relationship to affective disorders. Rabstein et al. [69] report an increased risk of breast cancer in night workers and AANAT polymorphisms. Zienolddiny et al. [70] also reported that variant SNPs in AANAT are associated with an increased risk of breast cancer in night shift workers. Sekine et al. [71] found a large number of AANAT polymorphisms in the Japanese population.

Pereira et al. [72] have suggested that excessive melatonin production in winter months may reduce tryptophan concentrations in the blood and thereby cause seasonal affective disorder. All of these reports indicate that it is important to understand the relationship between the activity of AANAT and melatonin in the blood and CSF, which is easy to compute in the model. Fig. 3 shows the model concentrations at steady state of *bmel* and *csfmel* as a function of the relative activity of AANAT as this activity ranges from 1 (day) to 29 (night). As expected, the concentrations rise as AANAT activity rises but note that the rise is not linear, but the slope is smaller at higher AANAT vales. This is because the depletion of 5*ht* partially counteracts the increase in activity of AANAT. Note that during the dynamic simulation in Fig. 2, the 5*ht* concentration drops dramatically at night.

3.3. The effect of bright light at night

It has been known since the early papers of Klein [33] that bright light at night causes the melatonin concentration in the blood to decrease rapidly. Since then, it has become clear that this not just an annoyance, but that exposure to light, especially bright light, at night may have serious health consequences. A number of epidemiological

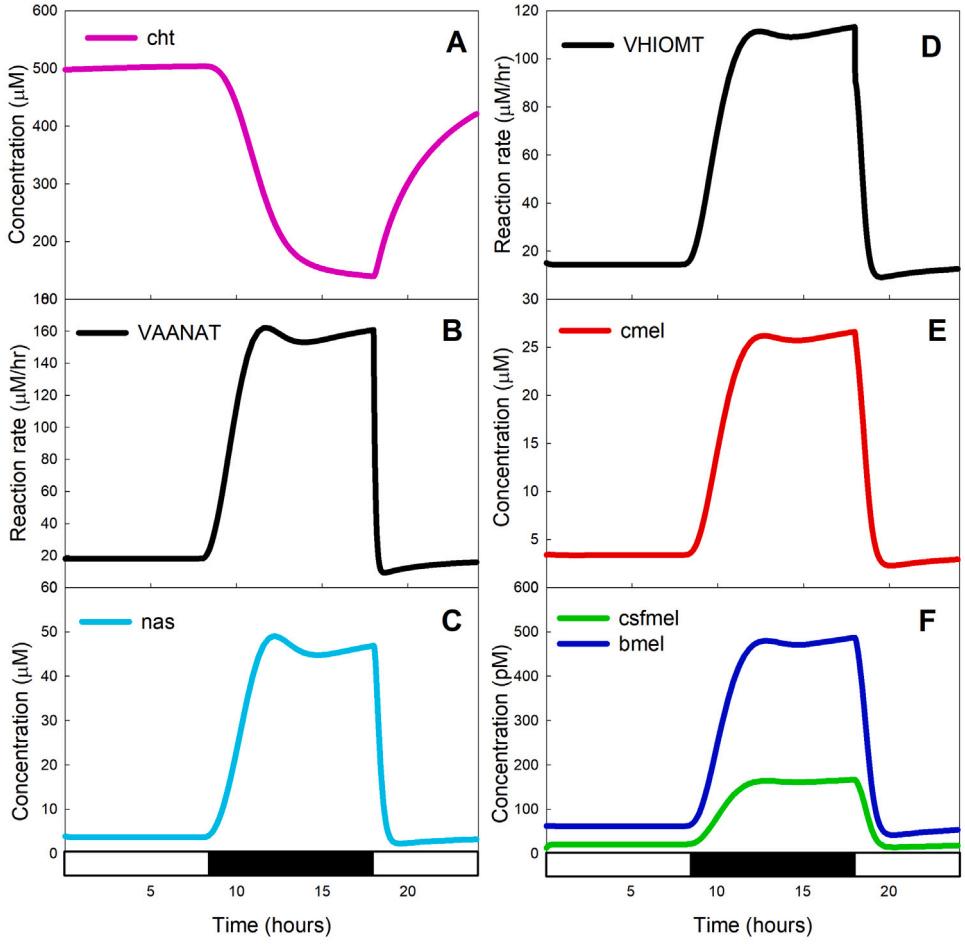

Fig. 2. Dynamic changes in melatonin synthesis. During the day, serotonin is very high in pineal cells but is drawn down to 150 μM at night (Panel A). The upregulation of AANAT by norepinephrine at night causes the velocity of the AANAT reaction to be eight-fold higher at night (Panel B). The product of the AANAT reaction is nas, whose concentration shows the same pattern (Panel C). The velocity of the HIOMT reaction (panel D) is driven partly by the modest increase in HIOMT activity but mostly by the increase in its substrate *nas*. Melatonin is produced by the HIOMT reaction (panel E) and has the same shape, as do blood melatonin and CSF melatonin (Panel F). In our model, Time = 0 is 12 noon.

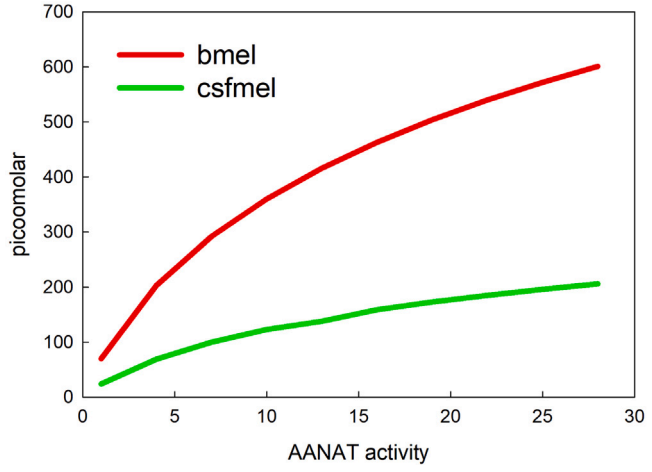

Fig. 3. Dependence on AANAT. The curves show the concentrations of *bmel* (red) and *csfmel* (green) as a function of the relative activity of AANAT.

studies have shown an association of bright light exposure at night with cancer, especially breast cancer [73]. Normal human blood pressure dips at night and this dip is attenuated by bright light, which may therefore affect cardio-vascular diseases [74,75]. Finally, melatonin has profound effects on the neuroendocrine-reproductive axis in mammals and therefore probably in humans [73,76]. Thus, the effect of bright light at night is an important area of biological and medical research, and it also provides an opportunity to test our model against clinical data.

Czeisler et al. [36] investigated the effect of bright light (for 90 min) in the middle of the night on blood melatonin in blind and normal subjects. Bright light inhibits the stimulation of the PVN by the SCN and thereby eliminates the stimulation of AANAT by norepinephrine. We model this by setting the increased nighttime stimulation of AANAT back to its daytime value for 90 min starting at 1 am. The model result can be compared to Czeisler's observations for normal subjects in Fig. 4.

The model curve in Panel B is very similar to the clinical curve in Panel A. No parameters were changed in the model; we used the same model that produced the curves in Figs. 2 and 3. The melatonin concentrations in the clinic and in the model decrease rapidly when lights are turned on and then increases rapidly when lights are turned off with similar slopes. We note that our model blood melatonin concentrations are approximately 500 μM, consistent with other clinical data but higher than the clinical curve in Fig. 3A.

3.4. Melatonin phase response curve

Exogenous melatonin is widely used to manage the symptoms of jet lag and sleep disorders, having been shown to entrain circadian rhythms in the SCN [60,77]. The ideal timing of melatonin depends on

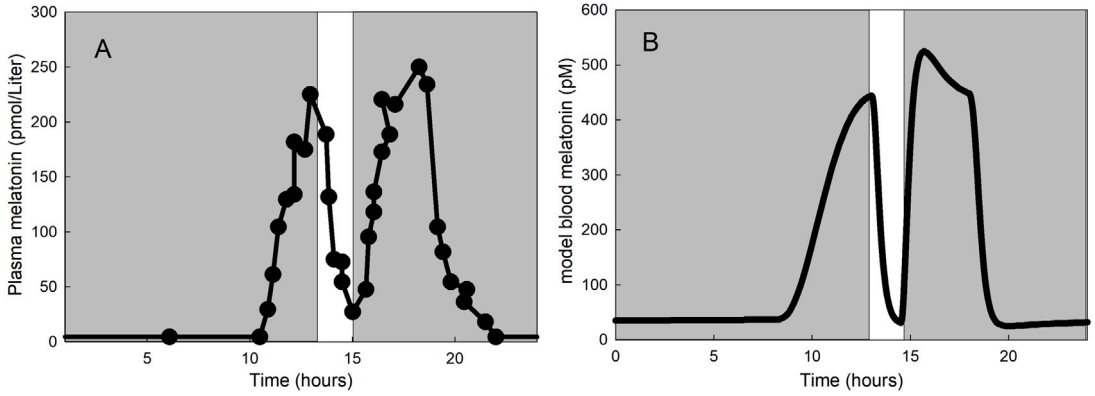

Fig. 4. The effect of bright light at night. Panel A shows the average blood melatonin concentration of six normal subjects exposed to bright light (white bar) for 90 min redrawn from [36]. Panel B shows the result of a similar experiment in the model where the activity of AANAT was set back to its low daytime value for 90 min starting at 1 am. As in our other simulations, night runs from 8 pm to 6 am. In our model, Time = 0 is 12 noon. See text for discussion.

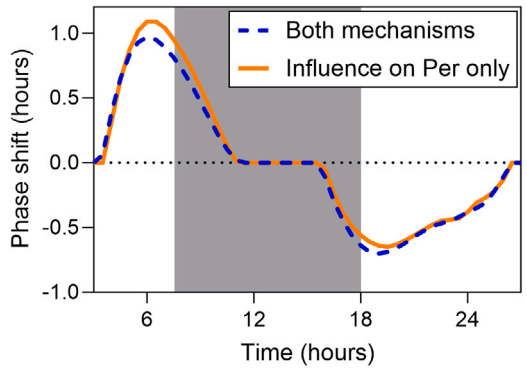

Fig. 5. Melatonin phase response curve (PRC). In the model, we administer melatonin daily at the same time each day for 49 timepoints between $t = 3$ and $t = 27$. Melatonin administration between $t = 3.5$ and $t = 11.5$ phase advances the circadian clock while melatonin administration between $t = 16$ and $t = 27$ phase delays the circadian clock. There is a "dead zone" in between when melatonin administration has no effect on the circadian phase. Removing the influence of melatonin on Bmal1 via ROR does not significantly change the shape of the PRC. In our model, Time = 0 is 12 noon.

its phase response curve (PRC) which plots the resulting phase shift for melatonin doses given at different times. Experimentally derived PRCs generally show that melatonin taken late afternoon or early evening phase advances circadian rhythms while melatonin taken late evening or early morning phase delays circadian rhythms [13,77–80], though the precise timing seems to depend on the dose [81]. It has also been observed experimentally that the melatonin PRC has a "dead zone" during the dark phase when exogenous melatonin has little to no effect on the phase of the circadian clock [78].

We couple our mathematical model for melatonin synthesis into our molecular clock model to understand how doses of melatonin temporarily influence circadian phase. It has been found that melatonin induces an increase in Per expression levels and this mechanism is likely important for phase-resetting [60]. There is also some evidence that melatonin influences the molecular clock by stimulating Bmal1 transcription, although the mechanism remains debated [61]. In our mathematical model, we incorporate both mechanisms. We add a melatonin signal to the equations for both P_1 and S. Then, we administer melatonin at different times of the day and measure the average phase shift in PER (P_2) over the next 24 h to obtain a PRC; see Fig. 5.

Surprisingly, we find that the influence of melatonin on Per alone explains the shape of the phase response curve found in experiments, with a "dead zone" in the middle of the dark phase when melatonin does not phase shift the molecular clock. We find that our model melatonin doses advance the molecular clock when administered between $t = 3.5$ and $t = 11.5$ and delay the clock when administered between $t = 16$ and $t = 27$. We additionally compute the PRC after removing the influence of melatonin on S, by setting $M_{total} = 0$ in Eq. (19). This does not significantly change the shape of the PRC; see Fig. 5. Our model results suggest that the phase-shifting effects of melatonin largely depend on its stimulation of Per transcription.

The exact timing depends on individual parameters. There is large inter-individual variability in melatonin secretion profiles [82] as well as individual differences in the response to exogenous melatonin [83], and the model can be used to study which parameters contribute to these inter-individual differences.

4. Discussion

No mathematical model can capture the full complexity of the biology that it seeks to understand and explain. Notably, our model does not include the details of the neural projections from the SCN to the PVN and from the PVN to the extracellular space in the pineal, releasing norepinephrine that upregulates AANAT and HIOMT. While it has been known for some time that clock genes are expressed in the pineal gland [84], how this local clock interacts with the signal from the SCN to affect oscillating melatonin levels is still being explored [85]. Secondly, gene expression levels between individuals vary on the order of 25% [86–88], and thus our model should be considered as a model for an average person, but not necessarily any particular individual.

The phase-resetting effects of light on circadian rhythms are well studied [89–91]. These models can be used to determine how long it takes the circadian clock to re-entrain during jet lag [92] and predict optimal times for light exposure to reset the circadian clock quickly [93]. Similar modeling approaches were used to study the phase-resetting effects of melatonin doses on circadian rhythms [27]. However, all of these studies use a simplified circadian model in the form of a single pacemaker. To our knowledge, our mathematical model is the first to describe the effects of melatonin doses on specific circadian genes and proteins in the molecular clock. In the model, we can explain the phase-shifting effects of melatonin on circadian rhythms as a consequence of the intracellular mechanism by which melatonin stimulates Per expression. We are even able to capture the "dead zone" of the melatonin PRC often observed in experiments [13] and not reproduced in other models [27].

Pineal melatonin hormone acts to integrate behavioral and physiological processes with respect to circadian timing through its effects on numerous peripheral systems [13]. Melatonin helps shape the circadian variation of energy metabolism, in part through its effects on the pancreas, liver, and white adipose tissue. In rodents, melatonin controls

clock gene expression in white adipose tissue, regulating the timing of lipolysis, lipogenesis, leptin production, and adipocyte proliferation [94]; this seems to be the case for humans as well [95]. It affects the circadian rhythmicity of pancreatic beta cells and may play a role in sensitizing these cells to glucagon-like peptide 1 (GLP-1) [96], causing the release of increased insulin. Melatonin also suppresses glucose production in the liver during the active period [13,97]. Melatonin appears to play a chronobiotic role in adrenal glands [98], cardiomyocytes [99], the retina [100], and likely does so in numerous other cell types. The model that we have developed accounts for the production of melatonin and cross-talk between the SCN and the pineal gland, and can be expanded to study the effects of melatonin on these other tissues.

The authors have studied sex differences in one-carbon metabolism [101,102] and glutathione metabolism [103] in the liver and explained large differences between men and menstruating females caused by androgens and estrogens. It is known that there are behavioral sex differences in circadian rhythms. Women tend to go to bed earlier and wake up earlier than men and are more likely to identify as morning types [104]. In addition, women seem to be more affected by night shift work than men, with an increased risk of work injury for women working nonstandard shifts for an extended period of time [105,106]. This suggests that androgens and estrogens may affect the pineal gland and the SCN. Indeed, early work showed that estradiol affects MAO in the pineal [107], protein synthesis and the activity of HIOMT [108], and that both estradiol and androgen affect pineal metabolism [109]. Later studies showed that the length of the melatonin signal drives the timing of reproduction in ewes [110] by affecting the frequency of LH pulses [111]. Much of this early work is reviewed in [112]. More recent work has emphasized the complicated crosstalk between the melatonin system and the hypothalamic-pituitary-gonadal axis [113, 114]. In addition, although most circadian research has involved only male animals [115], it has been known for decades that there are sex differences within the SCN, with human females having greater relative volume and rostrocaudal axis length [116]. The catalog of known sexual dimorphisms within the SCN continues to grow, including levels of expression of a peptide important for coupling SCN neurons [117], electrical properties (and therefore activity) of SCN neurons [118], and levels of androgen [119] and estrogen [120] receptors. Among the possible implications of these differences is that females may more readily respond to environmental [115] or central [121] cues that phase shift the SCN clock. In future work, the authors plan to extend the mathematical model in this paper to study sex differences in the pineal gland and the SCN.

CRediT authorship contribution statement

Janet Best: Conceptualization, Investigation, Methodology, Writing – original draft, Writing – review & editing. **Ruby Kim:** Conceptualization, Investigation, Methodology, Software, Writing – original draft. **Michael Reed:** Conceptualization, Investigation, Methodology, Software, Writing – original draft, Writing – review & editing. **H. Frederik Nijhout:** Conceptualization, Investigation, Methodology, Visualization.

Declaration of competing interest

The authors affirm that we have no conflicting or competing interests that could influence this work.

Data availability

The Matlab code used for this research is available by request from the authors.

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