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Structures and molecules involved in generation and regulation of biological rhythms in vertebrates and invertebrates

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Abstract. Melatonin from the retina and the pineal gland functions in neuroendocrine hierarchies. Photoreceptors – eyes and extraretinal – detect light. Oscillators – pineal and suprachiasmatic nuclei – act as pacemakers. Driven neuroendocrine rhythms carry temporal hormone signals throughout the body. Light controls melatonin: light sets the phase of the melatonin rhythm and determines the duration of melatonin synthesis. By these means, circadian rhythms (e.g. in locomotor activity and body temperature) and seasonal rhythms (e.g. in reproduction) are controlled.

Key words. Melatonin; rhythm; circadian; pineal; eye; light; suprachiasmatic nucleus.

Hierarchies

Physiological events are organized in temporal frameworks that include life cycles of development and rhythms. Melatonin has been implicated in both of these temporal processes. Melatonin has been proposed as having a role in development (e.g. onset of puberty)[52] and aging (e.g. lifespan)[35]. Melatonin has been implicated as a key player in circadian rhythms (e.g. locomotor activity, body temperature) and in seasonal cycles (e.g. reproduction).

In vertebrates, the role of melatonin in temporal organization takes place by the part it plays in neuroendocrine hierarchies. While various schemes have been proposed (fig. 1) these hierarchies generally involve a photoreceptor (e.g. the eyes), a driving oscillator (e.g. the suprachiasmatic nucleus), driven hormone rhythms (e.g. melatonin), and observed cycles (e.g. locomotor activity).

Photoreceptors

Since most circadian rhythms respond to light cues, such as the solar day-night cycle, finding the photoreceptors and tracing their connections has been key to understanding the hierarchies of organization. In mammals, the photoreceptor was the obvious one – the eyes. The connection by which the eyes convey information about light in the environment is less obvious. A retinohypothalamic tract (RHT), separate from the optic nerves, connects the retinas to the brain[34].

In birds, reptiles, amphibians, and fish, however, the photoreceptors turned out to be more surprising. In these species, blind animals detect light. They have extraretinal photoreceptors (ERR)[32]. With extraretinal photoreception, light entrains circadian rhythms (e.g. sparrow locomotor activity[49]) and mediates photoperiodic responses in reproduction (e.g. testis size in sparrows[31]). The location of the extraretinal light receptors has been elusive, but is believed to be somewhere in the brain.

There is redundancy in photoreceptors in some species – the eyes, pineal, and other extraretinal photoreceptors are all capable of mediating light to entrain circadian rhythms. There are multiple effects of light on circadian rhythms in vertebrates – light phase shifts and entrains circadian rhythms, light has direct (or masking) effects, light alters the period length. Evidence that light might cause these multiple effects by 'two input pathways with different absorption and temperature characteristics' has recently been proposed for the marine bioluminescent alga, *Gonyaulax*[41].

Oscillators

The usual technique for identifying an oscillator was ablation of the suspected structure by lesioning or surgery (fig. 2).

In mammals, the pacemaker, or driving oscillator, for the circadian rhythm was localized to the hypothalamus by Curt Paul Richter[42]. Using lesions, Stephan and Zucker[44] further refined its location to a pair of regions called the suprachiasmatic nuclei. The role of the suprachiasmatic nuclei (SCN) as a pacemaker was supported by the finding of glucose uptake rhythms[43]. The SCN also plays pacemaker roles in other vertebrate species (table).

The pineal gland was identified as a site of circadian oscillation when its removal from house sparrows resulted in arrhythmic activity[21] and body temperature rhythms[8]. The case for a pineal role in circadian regulation was further developed by the discovery of pineal light sensitivity and rhythms in the enzymes that synthesize melatonin (N-acetyltransferase or NAT, hydroxyindole-O-methyltransferase or HIOMT) and in melatonin itself (fig. 3). Investigators using transplants provided evidence that the sparrow pineal gland carried both the ability to generate a circadian oscillation and the phase information[51]. Experimenters who placed the avian

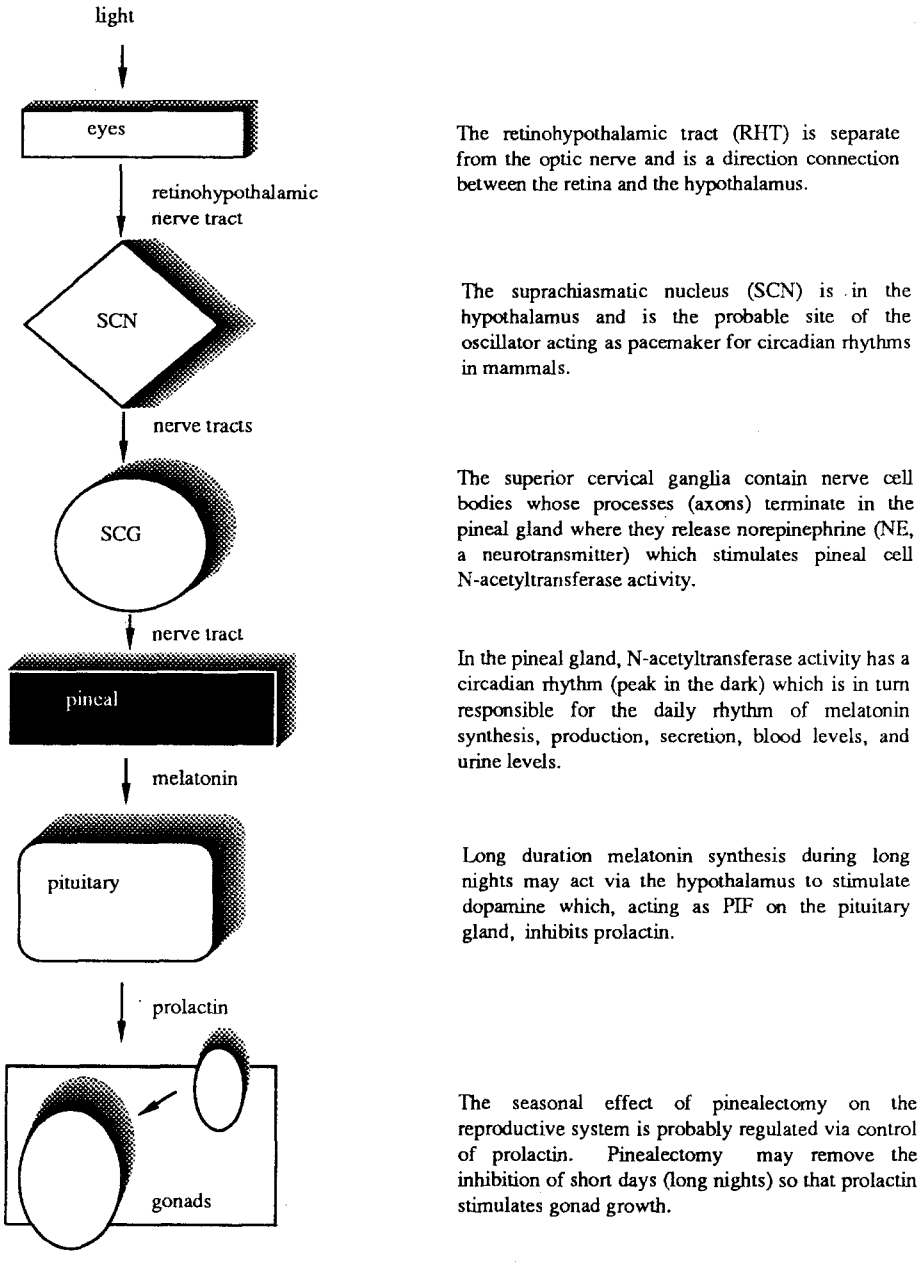

Figure 1. Regulation of the seasonal changes in testis size is shown as an example of a hierarchical scheme.

pineal gland in organ culture showed that the pineal had intrinsic ability to carry phase information[12], and the innate capacity to generate a cycle[11] with the enzyme, N-acetyltransferase. In organ cultures, the pineals of chickens[4] and lizards[33] and sparrows[47] secrete melatonin in a rhythmic fashion (fig. 4). However, in mammals, the pineal gland is not capable of oscillation; instead, its rhythms are driven by the SCN[44]. A pathway for light control of the gonads via melatonin can be suggested: light → eyes → suprachiasmatic nuclei → superior cervical ganglia → norepinephrine release across synapses in the pineal gland → activation of adenyl cyclase → increased cyclic AMP → increased N-acetyltransferase activity → increased duration of melatonin → decreased prolactin → smaller inactive gonads.

However, rhythmic melatonin secretion is not limited to the pineal gland. NAT and melatonin content rhythms are also in the retina[7]. The discovery of retinal melatonin synthesis presented a solution to the puzzle of why pinealectomy was ineffective in experiments in some species. It meant that the eyes themselves are also candidates for oscillators or roles in the hierarchies. And, in addition, a role for melatonin in retinal function – disc shedding, photoreceptor cell elongation, pigment migration, occular fluid regulation – has been suggested[5].

Eyes have also been proposed as oscillators in invertebrates, such as *Aplysia*[13]. Sea slugs, *Aplysia*, seem far removed from vertebrates. Nevertheless, the pacemaking systems in their eyes involve serotonin, the precur-

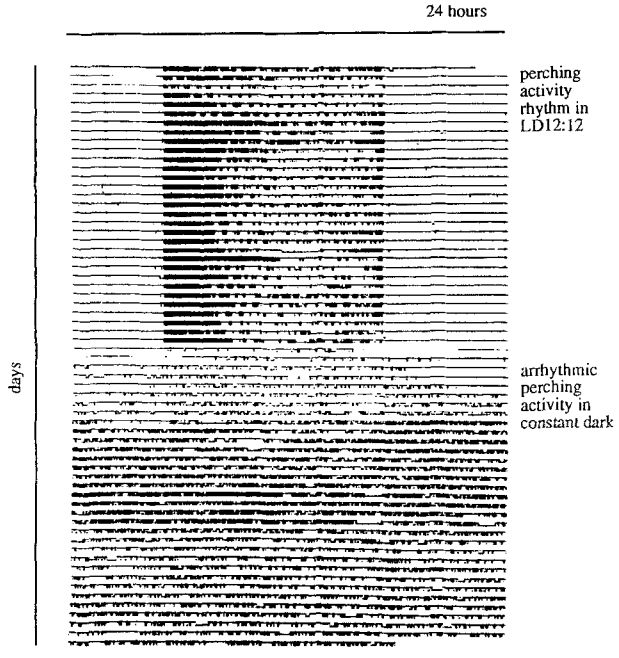

Figure 2. A pinealectomized sparrow (*Passer domesticus*) entrains to a light-dark cycle (LD12:12, activity during the light), but, in constant dark where its activity rhythm would normally persist, the rhythm is lost.

Pacemakers

SCN	Pineal	Eyes	Optic lobe	Brain
House sparrows	House sparrows	Pigeons	Roaches	Fruit flies
Java sparrows	Java sparrows	chickens	crickets	
Humans	Pigeons	Quail	Silk moths	
Hamsters	Chickens	Sea hare		
Rats	Quail	African toad		
Ground squirrels	Lizards			

sor of melatonin[16]. The oscillator has not been localized in plants, but some effects of light on plants are mediated by an indole relative of melatonin, indoleacetic acid, or auxin[2]. It would be interesting to determine whether light regulation of auxin plays a role in circadian organization of plants similar to light regulation of melatonin.

However interesting hierarchies of oscillations are in multicellular organisms, it should be remembered that many single-celled organisms are capable of circadian rhythms. *Gonyaulax* is one of these[46], and *Euglena* is another. These single-celled organisms contain all the machinery to generate circadian rhythms and have caused some invesigators to hypothesize that the cell cycle itself is involved[18].

In higher vertebrates, oscillators appear to be associated with the brain and the slave oscillations appear to be

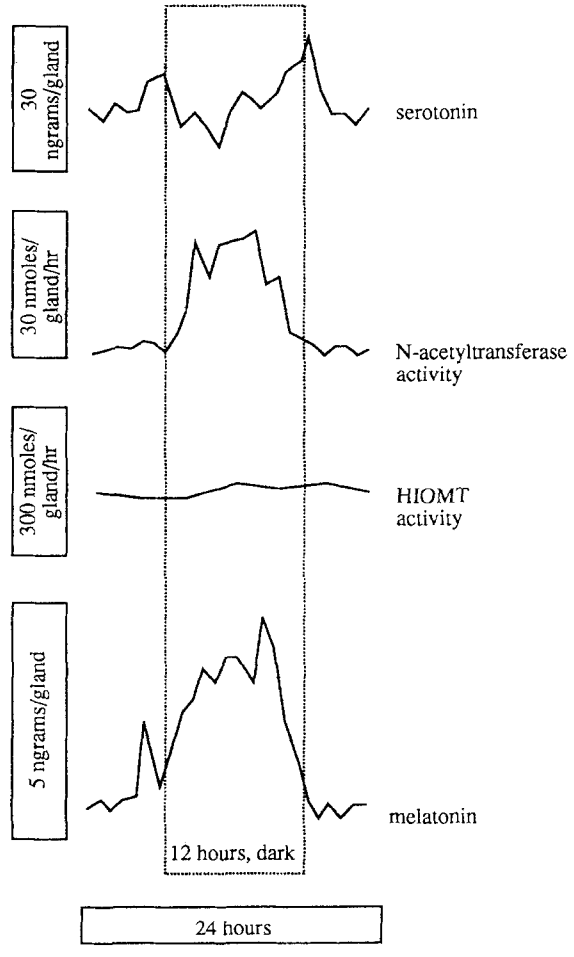

Figure 3. Daily changes are found in pineal enzymes and metabolites in young chickens (*Gallus domesticus*) housed in LD12:12. Pronounced daily cycles are found in serotonin (bimodal, with peaks at dawn and dusk), N-acetyltransferase (30-fold), and melatonin (10-fold).

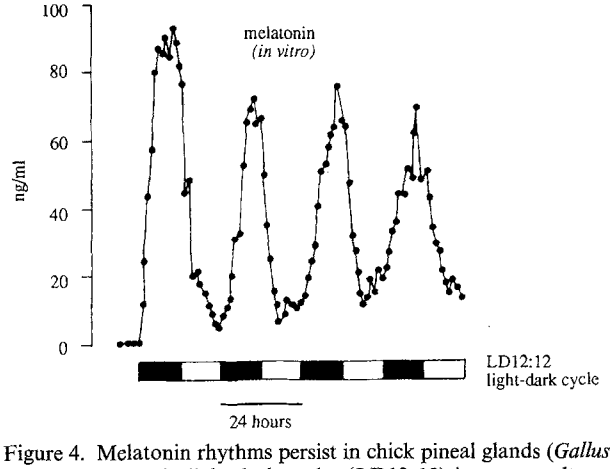

Figure 4. Melatonin rhythms persist in chick pineal glands (*Gallus domesticus*) kept in light-dark cycles (LD12:12) in organ culture. Data of Binkley and Tamarkin.

hormones secreted from endocrine glands. Most hormones that have been examined have pronounced daily cycles (fig. 5): cyclic AMP[28], CRF[22], osteocalcin[30], MSH[48], melatonin[39], TSH[14], PTH[29], insulin[19], ACTH and corticosteriod[27], aldosterone[15], epinephrine and

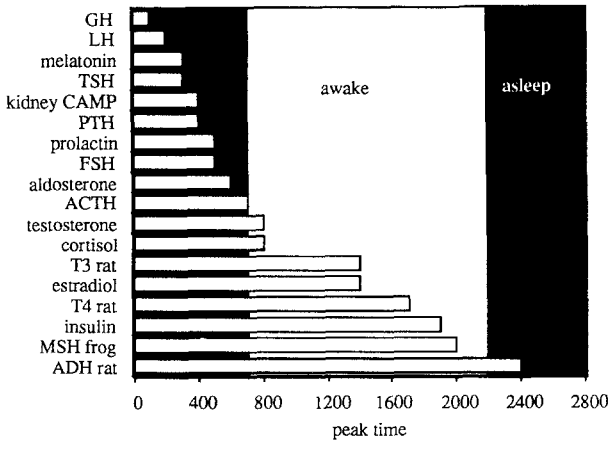

Figure 5. Peak times (right ends of bars) of hormone daily cycles scan 24 h in a sequential manner.

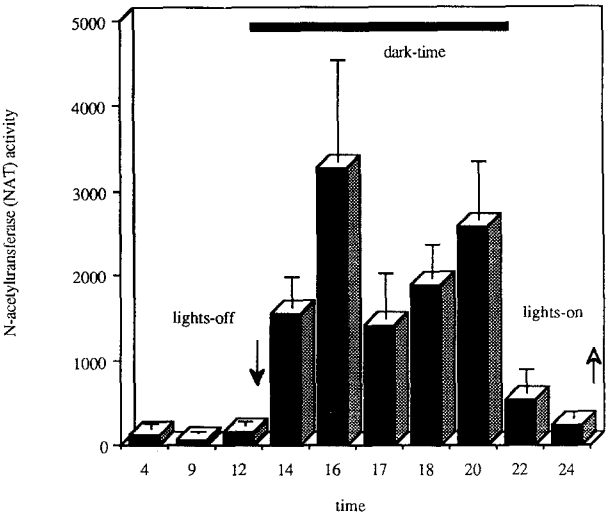

Figure 6. The daily rhythm of N-acetyltransferase (NAT) activity (pmoles/pineal/hr; here shown for the house sparrow, *Passer domesticus*) controls the daily cycle in melatonin production.

norepinephrine[45], LH and testosterone[23], FSH and estradiol[40], prolactin[19], and ADH[26]. The pineal is the principal endocrine gland that has been shown capable of generating oscillations in some species. However, in one report, the adrenal glands of hamsters were capable of generating cycles in organ cultures[1]. Adrenal steroids, in particular, are known to be secreted in cyclic fashion with peak hormone secretion early in the morning in vivo[27].

Melatonin, the circadian rhythm, light, and dark

The daily cycle of production of melatonin is controlled by the enzyme, N-acetyltransferase (NAT). So the activity of NAT determines, for the most part, the melatonin production.

NAT, an enzyme in the pathway for melatonin synthesis, was discovered to be rhythmic with activity at night that was thirty times the daytime activity in rats (fig. 6)[24]. The rhythm persists with a period close to 24 h in constant dark, DD (fig. 7)[6]. The rhythm is damped by constant light[38]. Circadian rhythms in general are well defined in light-dark cycles, persist or freerun in constant dark, and are suppressed by constant light. So the pineal provides a mechanism for these responses.

Light imposed during the dark-time causes a rapid plummet in NAT activity[25], decrease in melatonin[9], and increase in serotonin[37]. This means that NAT and melatonin are produced when dark is present. If dark is reimposed after a light pulse, NAT increases early in the night, but late in the night cannot reinitiate[10]. This provides a mechanism which can account for phase shifting of the circadian rhythm by light pulses[3]. After light pulses early in the subjective night, NAT reinitiates a new cycle, the rhythm is delayed. After light pulses late in the subjective night, NAT does not reinitiate, the period of low enyzme activity commences, and the rhythm is advanced. Suppression and reinitiation (or not) of NAT can explain the results of light break and resonance experiments which have been used to demonstrate the Bunning hypothesis for photoperiodic control (e.g. when light imposed with respect to the organism's circadian clock determines whether the photoperiod is perceived as long or short).

Not only do the responses of NAT explain phase shifting, they also explain how photoperiod can be measured. At dusk (or lights-out in the laboratory), NAT initiates and remains high until it has run its preprogrammed night-time duration. If light appears early, the NAT is suppressed. So in short photoperiods, NAT is high for a long time; in long photoperiods NAT is high for a short time (fig. 8). Thus the length of the night is measured and is translated into a signal via melatonin secretion. The site of melatonin action has been sought. Investigators, using techniques to measure melatonin receptors, have focussed on the hypothalamus and the gonads as sites of melatonin action.

There is an additional refinement to the seasonal control of melatonin. The enzyme, hydroxyindole-O-methyltransferase is also subject to regulation by light and dark[36]. This means that HIOMT can also be involved in the seasonal modulation of melatonin secretion and may be rate limiting at some times of year in some species[50].

Is there a general model?

A general model for the control of circadian oscillations appears to involve light, light detector(s), oscillator(s), and, in some species, hormones. But there are some puzzles.

In formulating general models, one puzzling aspect is the apparent redundancy of photoreceptors, oscillators, and melatonin synthesis sites which occurs in vertebrates. Lizards have a plethora of photoreceptors: eyes,

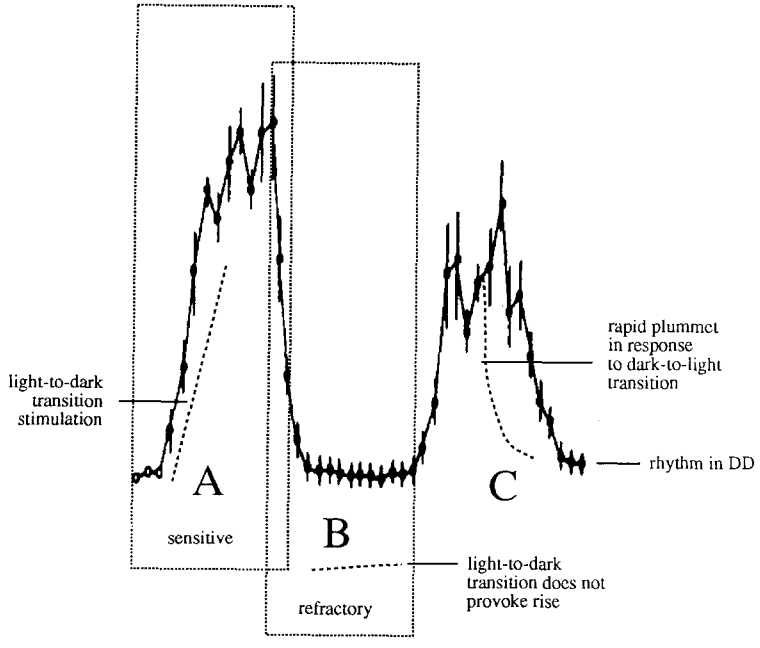

Figure 7. The daily rhythm of N-acetyltransferase activity (here shown for chickens, *Gallus domesticus*) persists (freeruns) in constant darkness and therefore qualifies as a true circadian rhythm. Responses of the enzyme activity to light and dark (broken lines) are stimulation (or lack of inhibition) by darkness during a sensitive phase, failure of darkness to provoke an activity increase during a refractory phase, and a rapid plummet of enzyme activity in response to light during the sensitive phase if NAT activity is initiated.

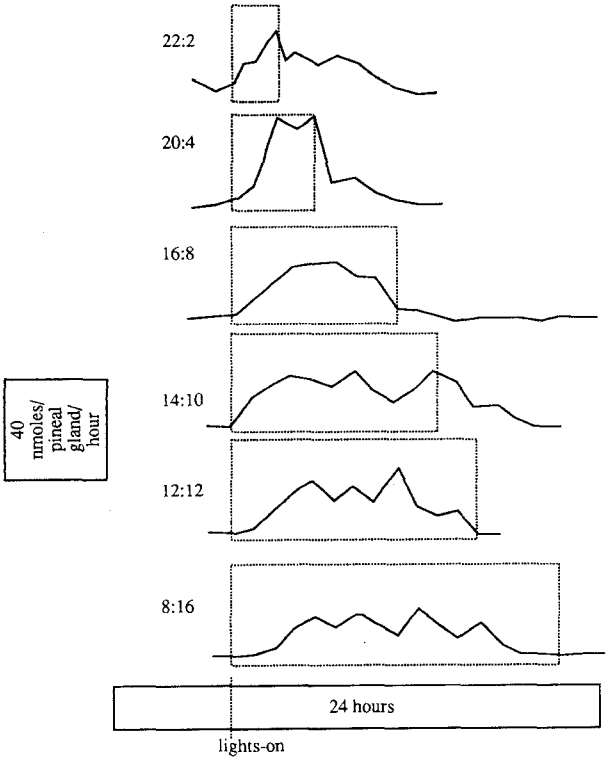

Figure 8. The amplitude and duration of the daily peak of N-acetyltransferase is modified by photoperiod (here shown for the chick, *Gallus domesticus*). In turn, the NAT activity controls the duration of melatonin synthesis. Thus, the duration of dark is translated into an endocrine signal.

pineals, and extraretinal photoreceptors. Birds seem to have more oscillators than they need: SCN and pineal. Melatonin production takes place in more than one location. The question remains, why is there redundancy?

Enzyme rhythms (e.g. N-acetyltransferase activity) seem likely candidates for the means by which oscillation information is converted to physiological signal (e.g. a hormone such as melatonin). NAT provides one example in vertebrates, but there are also rhythmic enzymes in nonvertebrates (e.g. light-time superoxide dismutase activity in *Gonyaulax*[16]). So enzyme rhythms are good candidates for having roles in a general model. An area where explanation is required for a general model is the means to achieve the difference between nocturnal and diurnal behavior. Melatonin secretion and NAT activity are high at night in all species so far studied, yet some of those species are nocturnal (e.g. rats, hamsters) while others are diurnal (e.g. sparrows, chickens, humans).

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