

## The pineal and melatonin: Regulators of circadian function in lower vertebrates

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**Summary.** The pineal has been identified as a major circadian pacemaker within the circadian system of a number of lower vertebrates although other pacemaking sites have been implicated as well. The rhythmic synthesis and secretion of the pineal hormone, melatonin, is suggested as the mechanism by which the pineal controls circadian oscillators located elsewhere. Both light and temperature cycles can entrain the pineal melatonin rhythm. The pineal, therefore, acts as a photo and thermoendocrine transducer which functions to synchronize internal cycle with cycles in the environment. A model is presented which portrays the pineal as a major component of a 'multioscillator' circadian system and which suggests how these multiple circadian clocks are coupled to each other and to cycles of light and temperature in the external world.

**Key words.** Pineal; melatonin; circadian rhythm.

Organisms, from unicellulars to vertebrates, are structured in time as well as in space. Many, if not most, biochemical, physiological and behavioral parameters exhibited by organisms show daily fluctuations. Significantly, most of these daily rhythms will persist under constant conditions, with periods near 24 h in length, showing that they are driven by an internal daily or 'circadian clock'. Under natural conditions these rhythms are 'entrained' by environmental stimuli (such as light and temperature) so that the 'right' events occur at the 'right time of day'.

The use of the term 'circadian clock' does not mean that a single discrete 'clock' is responsible for driving all of an organism's daily rhythms. In recent years it has become appreciated that multicellular organisms are multi-oscillator in nature; that is, more than one circadian clock may exist within a single organism. Normally, however, all of an organism's multiple circadian rhythms exhibit fixed phase relationships with each other under both entrained and freerunning conditions. Accordingly, when multiple clocks exist within an individual organism, they must be coupled together in some manner.

Several areas have been implicated as being important to circadian organization in vertebrates; these include the pineal organ, the suprachiasmatic nuclei (SCN) of the hypothalamus, and the lateral eyes. Interesting similarities, as well as significant differences, seem to exist between species in the relative roles that these areas play within an animal's circadian system.

Among lower vertebrates most of the studies to date have focused on the role of the pineal organ. Embryologically, the pineal arises as an evagination of the roof of the diencephalon and, in lower vertebrates, the pineal is situated on the brain's surface and remains attached to the brain by a stalk<sup>26, 36, 45</sup>. Pineal organs are present in virtually all vertebrates and, in addition, a second component of the pineal 'system', generally termed a parapineal organ, is often present in lower vertebrates which arises from a more rostral differentiation of the diencephalic roof<sup>26, 36, 45</sup>. During development the parapineal component (called a frontal organ in anuran am-

phibians or a parietal eye in lizards) migrates to a position close to the roof of the skull. Parapineal organs are absent in birds, mammals, and in some lower vertebrates (such as snakes and toads).

The pineal organs of lower vertebrates typically contain several types of cells including photoreceptors and nerve cells (Falcón and Collin, this issue)<sup>26, 36, 45</sup>. The photoreceptive cells resemble the photoreceptors of the lateral eyes and have been found in petromyzontides, fish, amphibians, chelonians, and lacertilians. The photoreceptive cells may possess a well-defined outer segment with stacked lamellar disks as well as an inner segment and a cell body with a synaptic pedicle which establishes contact with one or more secondary neurons, or they may be more 'rudimentary' in appearance and exhibit disorganized or rudimentary outer segments and lack contact with secondary neurons. Rudimentary photoreceptors are characteristic of the pineal organs of chelonians and lacertilians. The pineal photoreceptors are hypothesized to be the precursors of the (secretory) pinealocyte which is the predominant cell type of ophidians, birds, and mammals.

The principal cell type in the parapineal organs of lower vertebrates is also photosensory in appearance. The ultrastructural evidence of photoreception by the parapineal (and the pineal) organs of lower vertebrates is supported by electrophysiological studies<sup>26</sup>. In general, pineal organs show an achromatic electrical response to illumination whereas parapineal organs are often capable of wavelength discrimination (chromatic response). In fish, amphibians and reptiles, nerve cells found within the pineal and parapineal organs project axons carrying photic information to pretectal and tegmental centers<sup>26, 45</sup>. From lower to higher vertebrates there is a definite phylogenetic trend toward a decrease in the intrapineal neurons and their pinealofugal fibers. Also, whereas pinealopedal (sympathetic) innervation, originating in the superior cervical ganglia, has been well documented in birds and mammals, pinealopedal innervation in lower vertebrates has been poorly studied. The pineals of some lower vertebrates appear to contain sym-

pathetic (efferent) nerves but the origin of these nerves is uncertain.

Despite the photosensory appearance of the main cell type in the pineals of lower vertebrates, ample evidence exists that these cells are also capable of considerable biosynthetic activity. For example, immunohistochemical and autoradiographic studies suggest that the pineal photosensory-type cells are the sites of synthesis of indoleamines, including melatonin (Falcon and Collin, this issue)<sup>15, 25, 67</sup>.

### Multiple photic inputs to the circadian system

Removal of the eyes does not prevent entrainment (synchronization) of the circadian system by 24 h light-dark cycles in any of the nonmammalian vertebrates examined so far<sup>1, 2, 4, 12, 24, 33, 51, 59, 61, 62</sup>. Extraretinal photoreceptors, therefore, exist in fish, amphibians, reptiles and birds which are fully capable of mediating entrainment. The photosensory capacities of the pineal systems of lower vertebrates have made them obvious candidates as the extraretinal photoreceptors. In a variety of fish and reptiles, however, removal of the pineal organ, or the parapineal organ, failed to abolish entrainment in blinded animals<sup>33, 59, 61, 62</sup>. It has been suggested that the frontal organ of frogs may be the extraretinal photoreceptor mediating entrainment since removal of the frontal organ was reported to abolish entrainment of blinded green frogs (*Rana clamitans*)<sup>2</sup>. The small sample size and the 'noisiness' of the activity rhythm, however, precludes any firm conclusions about the role of the frontal organ in *R. clamitans*. In another anuran, *Xenopus laevis*, entrainment of the activity rhythm of blinded *Xenopus* to LD cycles was unaffected by removal of the frontal organ<sup>4</sup>. Although these data show that entrainment can persist after removal of the eyes and pineal system – showing the universal existence of extrapineal-extraretinal receptors in lower vertebrates – the conclusion that pineal photoreception is not involved in mediating photic influences on the circadian system is unwarranted. In view of the major role of the pineal organ in circadian system in some vertebrates (see below), and in view of the fact that light can directly affect pineal biochemistry<sup>11, 20, 28, 37, 38</sup>, it seems probable that direct pineal photoreception is important to circadian organization, at least in some lower vertebrates.

The nature of the role of extraretinal photoreceptors (ERRs) and the eyes as photic inputs to the circadian system of lower vertebrates has been poorly studied. The (extrapineal) ERRs have been localized to the brain in fish, amphibians and reptiles, but their location(s) within the brain are not known<sup>1, 61, 62</sup>. Several observations suggest that the lateral eyes have a photic input to the circadian system of lower vertebrates including: a) Removal of the eyes of lizards (*Sceloporus olivaceus*) entrained to very dim LD cycles can cause them to freerun<sup>61</sup>. b) Blinding affects several light-dependent cir-

cadian properties in the lizard *Sceloporus occidentalis* including range of entrainment, rate of reentrainment to shifted LD cycles, and the sensitivity to light pulses<sup>55</sup>. c) In trout *Salvelinus fontinalis*, entrainment to natural LD cycles is weaker in blinded fish at certain times of year<sup>13, 14</sup>. d) In the lake chub, *Couesius plumbeus*, blinded fish have a more restricted spectral sensitivity and a higher energy requirement for entrainment than sighted fish<sup>33</sup>.

One important question that arises is: Why do multiple photic inputs to the circadian system of lower vertebrates exist? The fact that ERRs exist, in addition to the lateral eyes, suggests that the different photoreceptors may be processing different kinds of photic information. This idea is supported by studies in lizards in which it has been shown that the eyes and ERRs are extracting different kinds of information from the photic environment. Diurnal animals typically exhibit a shorter freerunning period when exposed to constant light (LL) than when exposed to constant dark (DD). This empirical generalization has been termed 'Aschoff's Rule'. The lizard *Lacerta sicula* obeys Aschoff's Rule when sighted but, after blinding, the lizard's freerunning period is unaffected when the lizard is switched from DD to LL<sup>61</sup>. Blind *L. sicula*, however, are still entrainable by LD cycles. These data show that the eyes mediate the continuous (parametric) effects of light on the velocity of the circadian oscillator but ERRs can mediate entrainment via the light to dark (transitional or non-parametric) effects of light. Clearly, in this species, different photoreceptors (eyes versus ERRs) are involved in extracting different kinds (parametric versus non-parametric) of photic information. Other species of lizards, however, can obey Aschoff's Rule even after blinding showing that the parametric effects of light can be mediated via ERRs in these species<sup>51, 61</sup>. The reasons for these differences among species are unknown but may well be related to the particular ecological niches to which these animals have evolved.

### Pineal as a pacemaker

A major role for the pineal within the circadian systems of lower vertebrates is revealed by observing the effects of pinealectomy on the freerunning activity rhythms (fig. 1). Pinealectomy can cause: 1) marked changes in the freerunning periods of fish (e.g., *Couesius plumbeus*, *Lota lota*, *Catostomus commersoni*) and lizards (e.g., *Sceloporus olivaceus*, *Sceloporus occidentalis*)<sup>31, 32, 34, 50, 51</sup>, 2) splitting of the activity rhythm into two major circadian components in a fish (*Catostomus commersoni*) and a lizard (*S. olivaceus*)<sup>30, 50</sup>, or 3) arrhythmicity in lizards (e.g. *Anolis carolinensis*, *S. olivaceus*, *S. occidentalis*) and fish (e.g. *Heteropneustes fossilis*)<sup>19, 50, 51, 53</sup>. Pinealectomy of the lizard *S. occidentalis* may also cause a major distortion of the phase response curve to light pulses<sup>52</sup>. Note that a given species may exhibit more than one kind of response to pinealectomy.

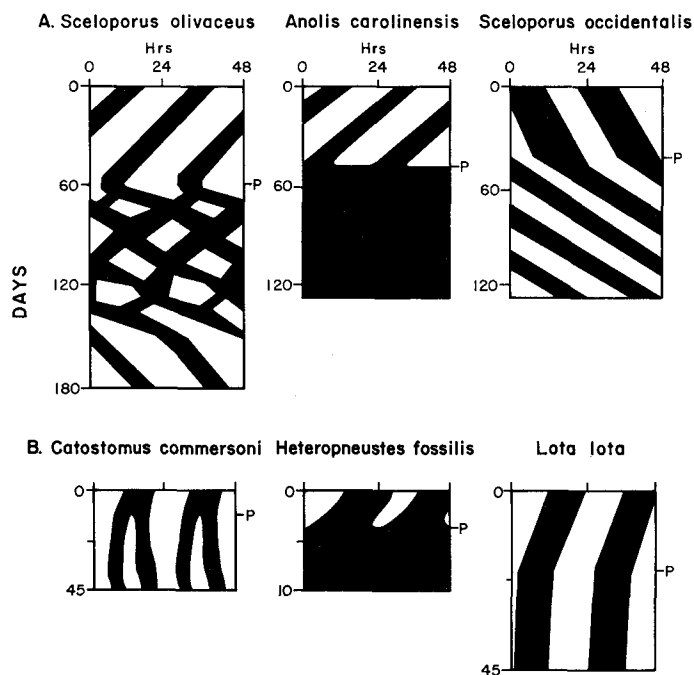


Figure 1. Effects of pinealectomy (P) on the circadian activity rhythms of several species of lizard (A) and fish (B). Pinealectomy has various effects including splitting of the activity pattern into two components (*S. olivaceus*, *C. commersoni*), arrhythmicity (*A. carolinensis*, *H. fossilis*), and period changes (*S. occidentalis*, *L. lota*). The original records from which these patterns were generated can be found in: (50, *S. olivaceus*), (53, *A. carolinensis*), (51, *S. occidentalis*), (30, *C. commersoni*), (19, *H. fossilis*), (32, *L. lota*).

These kinds of results are compatible with the hypothesis that the pineal can act as a pacemaker within a multi-oscillator circadian system. Obviously, circadian oscillators must also exist outside the pineal since, in some lizards and fish, rhythmicity can persist after pinealectomy. Both the inter- and intraspecies differences in the effects of pinealectomy could be explained by individual variations in the strength of coupling among these extrapineal circadian oscillators. For example, if coupling among extrapineal oscillators is weak (or absent) pinealectomy will cause splitting or arrhythmicity. If the extrapineal oscillator remain coupled following pinealectomy, a change in the period of the activity rhythm will be observed.

The pineal hormone, melatonin, appears to play an important role in the circadian system of lower vertebrates. Immunohistochemical and autoradiographic studies suggest that the pineal photoreceptive cells are the likely sites of melatonin production (Falcón and Collin, this issue)<sup>15, 25</sup>. Melatonin is highly lipid soluble and appears to be rapidly secreted into the blood by passive diffusion. Pineal melatonin production is rhythmic; melatonin levels at night are higher than those occurring during the day<sup>17, 20, 38, 54, 63</sup>. In higher vertebrates the daily rhythm in melatonin is believed to result from a rhythm in activity of the rate-limiting enzyme N-acetyltransferase (NAT), which converts serotonin to N-acetylserotonin.

The terminal enzyme in the melatonin synthesizing pathway, hydroxyindole-O-methyltransferase (HIOMT), is not believed to contribute to melatonin rhythmicity in higher vertebrates since its activity remains relatively constant throughout the day (Sugden, this issue). However, in some lower vertebrates (e.g., lamprey, *Lampetra planeri*; salmon, *Oncorhynchus tshawytscha*; lizard, *Lampropholis guichenoti*), but not others (e.g., pike *Esox lucius*), HIOMT activity or amount can also show significant daily (and seasonal) fluctuations<sup>8, 9, 16, 25, 29</sup>. The contribution of NAT versus HIOMT activity to melatonin rhythmicity in these cases has not been well studied. A role for melatonin in circadian organization is shown by the fact that: a) continuous melatonin administration can elicit period changes or arrhythmicity in some lizards<sup>51</sup>, b) daily melatonin injections can entrain the activity rhythm of the lizard *S. occidentalis*<sup>60</sup>, c) a phase response curve to melatonin exists in a lizard (*S. occidentalis*)<sup>56</sup>, and d) melatonin pulses can phase-shift celestial orientation in the tiger salamander, *Ambystoma tigrinum*<sup>3</sup> (accurate celestial orientation relies on the circadian clock to compensate for the apparent movement of celestial cues).

These data suggest that the daily rhythm of melatonin secretion may be the mechanism by which the pineal communicates with the rest of the circadian system. The entrainment of circadian clocks by entraining stimuli such as light (or melatonin) requires that the circadian clock exhibit a diurnal change in sensitivity to that stimulus; that is, a phase response curve to that stimulus must exist<sup>43</sup>. All known phase response curves are qualitatively similar. If it is hypothesized that the phase response curve to melatonin differs (i.e., quantitatively) among extrapineal clocks, the daily melatonin pulse could, at least theoretically, not only *entrain* these clocks but also exert *phase control* over them so that the myriad overt rhythms they control (i.e., locomotor activity, hormone levels, enzyme activities) would occur at the appropriate time of day.

In vitro studies of pineal melatonin secretion have been conducted in fish and lizards (fig. 2). In the trout *Salmo gairdneri* melatonin production is cyclical under 24-h LD cycles, showing that the organ cultured pineal is capable of direct photoreception, but no rhythmicity is observed in DD<sup>20</sup>. Similarly, in the iguanid lizard *Dipsosaurus dorsalis* a rhythm is seen in organ cultured pineals exposed to LD but not in pineals held in DD<sup>28</sup>. In another iguanid lizard, *Anolis carolinensis*, however, a temperature compensated rhythm of melatonin secretion persists for up to 10 circadian cycles in DD; this rhythm is directly entrainable by LD cycles<sup>38</sup>. A circadian rhythm in melatonin production in vitro is also seen in the iguanid lizard *S. occidentalis* in DD<sup>37</sup>.

The role of potential neural inputs to the pineals of lower vertebrates has not been studied. Efferent (sympathetic) nerve terminals have been seen but their origin and function is unknown. Speculatively, these nerves could serve

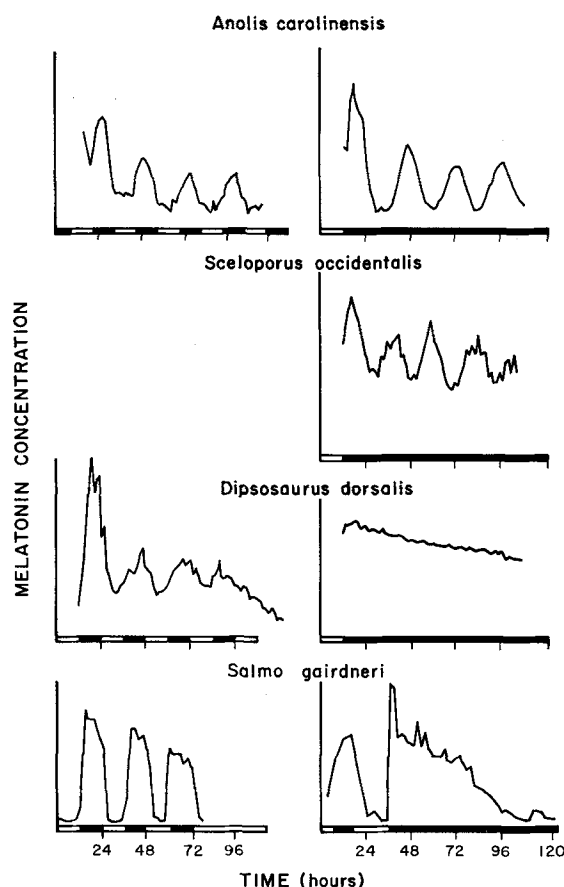


Figure 2. The in vitro pattern of melatonin secretion from individual pineal glands of several species of iguanid lizard (*A. carolinensis*, *S. occidentalis*, *D. dorsalis*) and one species of fish (*S. gairdneri*) held in either 24-h LD cycles (left panels) or in DD (right panels). The figures are redrawn from the following sources: (38, *A. carolinensis*), (37, *S. occidentalis*, courtesy The Ciba Foundation), (28, *D. dorsalis*, courtesy D. Janik), (20, *S. gairdneri*, courtesy Academic Press).

as routes by which extrapineal pacemakers are coupled to the pineal or they might be routes by which light perceived by the eyes or ERRs could influence the pineal. Also unstudied is the role of neural outputs from the pineal (or parapineal). These afferent nerves are undoubtedly transmitting photic information to pretectal and tegmental centers<sup>26,36</sup>, but the role of these photic inputs (if any) within the circadian system is unknown.

#### The pineal: A photothermoendocrine transducer

The pineal can function as a transducer of both thermal and photic information in poikilotherms. Studies have shown that either light or temperature cycles can entrain the pineal (or plasma) melatonin rhythm and aspects of the light or temperature cycles (such as the duration of the dark and light portions of the LD cycles or the amplitude of the temperature cycle) can influence aspects of the pineal melatonin rhythm (such as its amplitude, phase, or duration)<sup>18,21,22,54,58,63-65</sup>. When presented together, an interaction between light and temperature

cycles on the pineal (or plasma) melatonin rhythm can be observed<sup>17,18,22,58</sup> (fig. 3). The annual changes in the daily melatonin rhythm that are observed in animals exposed to natural conditions (fig. 4) undoubtedly reflect the interactive effects of both light and temperature on pineal melatonin synthesis. Light and temperature can undoubtedly *directly* affect the pineal melatonin rhythm, as revealed by in vitro studies<sup>20,28,38</sup>, but the possibility that photic (or thermal) inputs can reach the pineal indirectly from other brain areas cannot be discounted.

One possible way in which light and temperature may control the phase, amplitude, or duration of the pineal melatonin rhythm could reside in the putative *A. carolinensis* and *S. occidentalis* show that a circadian pacemaker must reside within their pineals which is capable of driving a rhythm of melatonin secretion. The results in *A. carolinensis* and *S. occidentalis* are compatible with the hypothesis that the pineal can act as a circadian pacemaker within a multioscillator circadian system. Such a role is also supported by the observation that pinealectomy in these two species induces period changes in the activity rhythm, or arrhythmicity, in DD<sup>51,53</sup>. Interestingly, pinealectomy of another iguanid lizard, the desert iguana *D. dorsalis*, has little effect on the freerunning

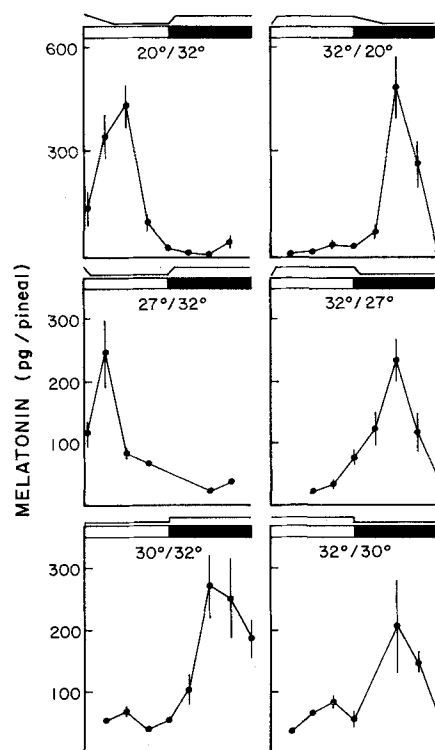


Figure 3. Interaction of light and temperature in the control of the pineal melatonin rhythm of the lizard *Anolis carolinensis*. The lizards were exposed to a temperature cycle in which the cool phase of the cycle occurred either during the day (left panels) or night (right panels) of a LD 12:12 light cycle. In the 'abnormal' light/temperature conditions (left panels: cool days/warm nights) the temperature cycles controlled the phase of the melatonin rhythm when its amplitude was 12°C (top), both light and temperature interacted to control phase when the amplitude of the temperature cycle was 5°C (middle), but the light cycle controlled phase when the amplitude of the temperature cycle was 2°C (bottom).

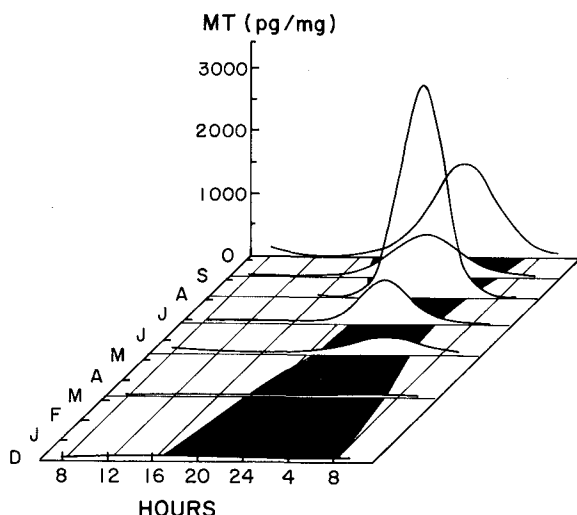


Figure 4. Annual changes in the daily rhythm of pineal melatonin content in the tortoise, *Testudo hermanni*, held under natural conditions of photoperiod and temperature (Adapted from 63, courtesy Academic Press).

activity rhythm and, as mentioned previously, the pineal of this species is also incapable of expressing a persistent circadian rhythm of melatonin production in vitro<sup>28</sup>. In those species in which a circadian rhythm of melatonin production is absent in vitro but a rhythm can be generated by exposing the pineal to LD cycles several possibilities can be entertained. 1) A circadian pacemaker may be absent in the pineal of these species but a rhythm in melatonin production can be exogenously generated by the periodic input of a photic, neural or hormonal signal. That is, the pineal is not capable of acting as a pacemaker but may be part of a driven system. 2) A pacemaker exists but the culture conditions are inadequate to allow expression of a rhythm. 3) A pacemaker exists but is not coupled to melatonin production.

Clearly, more studies are needed to determine, within the same species, the role of the pineal in the circadian system (i.e., by observing the effects of pinealectomy on the activity rhythm) and the behavior of the pineal in vitro. That is, are those species in which pinealectomy is ineffective in disturbing circadian rhythmicity the same species in which the pineal is incapable of expressing a circadian rhythm in melatonin secretion? The current data do, however, suggest that, in at least some species, the pineal can act as a circadian pacemaker and it is coupled to the rest of the system via the rhythmic secretion of the hormone, 'multioscillator' nature of the pineal itself. Dispersed cell cultures of chicken pineals, for example, can exhibit light-entrainable circadian rhythms in NAT activity showing that the pineal, itself, can be multioscillator in nature<sup>11</sup>. If a number of individual cells (i.e., the photosensory cells?) within the pineals of lower vertebrates possess circadian properties, the mutual phase relationships among these oscillators will determine the phase, duration, and amplitude of the cumulative output

of these cells (i.e., melatonin). Since both light and temperature can act as entraining agents for circadian clocks in poikilotherms, the way in which these stimuli entrain the multiple pineal oscillators will determine the phase, amplitude, and duration of the melatonin rhythm. Such a model could also be applied to pineals which do not exhibit persistent circadian rhythmicity in vitro (such as the trout or desert iguana) if the melatonin producing cells, although lacking persistent circadian rhythmicity, can act as damped oscillators which require daily stimulation by light or temperature to generate a melatonin rhythm. In this way the non-autonomous pineal could still act as a photothermal transducer but it would be expected that the role of the pineal within the total circadian system would be less.

The effect of light and temperature cycles on the pineal melatonin rhythm, therefore, offers a mechanism by which these stimuli can entrain the circadian system appropriately. That is, aspects of the pineal melatonin profile (i.e., phase, duration) directly reflect ambient photic and thermal conditions. The pineal melatonin rhythm, in turn, can phase extrapineal oscillators appropriately so that the overt rhythms they drive occur at the 'right time of day'.

One feature of the response of the pineals of at least some lower vertebrates to light is remarkably different from that observed in birds and mammals. In higher vertebrates light pulses presented at night cause a rapid (within minutes) fall in pineal melatonin levels<sup>47</sup>. This fall may not represent a 'direct' response to light (which in mammals is perceived exclusively via the eyes) but may reflect the way light shifts the phase of the circadian clocks driving NAT activity<sup>27</sup>. That is, light may cause a rapid shift of the phase of the clock to a phase at which it does not call for melatonin synthesis. In lower vertebrates, however, night breaks can depress melatonin levels in some (e.g. trout, *Salmo gairdneri*) but not in others (e.g. lizard *A. carolinensis*; turtle, *Terrapene carolina triunguis*)<sup>20, 57, 65</sup>. In the pike (*Esox lucius*) night breaks induced inactivation of roughly half of the maximal NAT activity indicating the existence of one photolabile and one photostable enzymatic component<sup>16</sup>. Clearly, additional studies are needed to determine the basis of the different kinds of response to night breaks – are these differences due to different forms of the NAT enzyme or to the way in which light pulses shift the phase of the oscillators driving the melatonin rhythm? Also, what is the adaptive value of the different kinds of response of pineal melatonin to night breaks?

#### Extrapineal melatonin production

Although it was originally believed that melatonin was a unique pineal product since HIOMT activity was thought to be uniquely localized in pineal tissue, it is now recognized that some extrapineal sites can generate melatonin<sup>46</sup>. In at least some lower vertebrates melatonin

synthesis can occur in the eyes, the parapineal organ, and possibly the Harderian glands<sup>17, 36, 41, 46, 66–68</sup>. Although it has been shown that blood levels of melatonin in higher vertebrates reflect mainly pineal melatonin secretion<sup>46, 67</sup> comparable studies in lower vertebrates are scarce<sup>21, 23, 28</sup>. In some species, such as the trout, the tiger salamander, and the desert iguana, pinealectomy does not completely eliminate blood-borne melatonin<sup>21, 23, 28</sup>. Clearly, in those species in which melatonin is important to circadian organization the potential exists for extrapineal melatonin-producing sites to play a role within the circadian system.

#### Extrapineal pacemakers

**SCN.** In mammals a host of studies have shown that the SCN of the hypothalamus are the site of a neural circadian pacemaker which drives many (if not all) of the animals' circadian rhythms<sup>39</sup>. Although fewer in number, available evidence suggests that the SCN may play a similar role in birds as well<sup>49</sup>. A direct retinohypothalamic (RH) tract to the SCN is the major route by which lighting information reaches the mammalian SCN<sup>39</sup>. In lower vertebrates, nuclei presumed to be homologous to the mammalian and avian SCN have been described, as well as the existence of a RH pathway<sup>28, 44, 48</sup>. However, only a couple of studies have examined the role of the hypothalamus in circadian organization in lower vertebrates. Surgical ablation of the optic tectum, the anterior portions of the telencephalon, and the dorsal portions of the telencephalon-diencephalon of the hagfish, *Eptatretus burgeri*, failed to affect the freerunning activity rhythm<sup>40</sup>. However, ablation of the ventromedial part of the telencephalon-diencephalon, which included the hypothalamus, caused hagfish to become arrhythmic in DD<sup>40</sup>. Interestingly, the hagfish is one of the most primitive vertebrates and it is one of the few animals in which a pineal organ is reported to be absent. Even if there is 'pineal equivalent' tissue in the dorsal diencephalon of the hagfish, it does not seem to have a pacemaking function since removal of the dorsal portions of the diencephalon did not abolish the circadian activity rhythm<sup>40</sup>. Lesions of the SCN of the desert iguana, *D. dorsalis*, abolish the circadian activity rhythm<sup>28</sup> (fig. 5).

The data from lower vertebrates, although sparse, suggest that the suprachiasmatic area of the hypothalamus is important for circadian organization and may act as a neural circadian pacemaker. The SCN, therefore, may be a site for at least some of the extrapineal pacemakers that exist in lower vertebrates.

**Eyes.** A small but growing body of evidence suggests that the eyes of some vertebrates may play a role within the circadian system which is not merely a photosensory one. There is some evidence that the eyes may be the loci of circadian pacemakers. Numerous studies have demonstrated that events within the eyes of lower vertebrates, such as photomechanical movements of rods and cones,

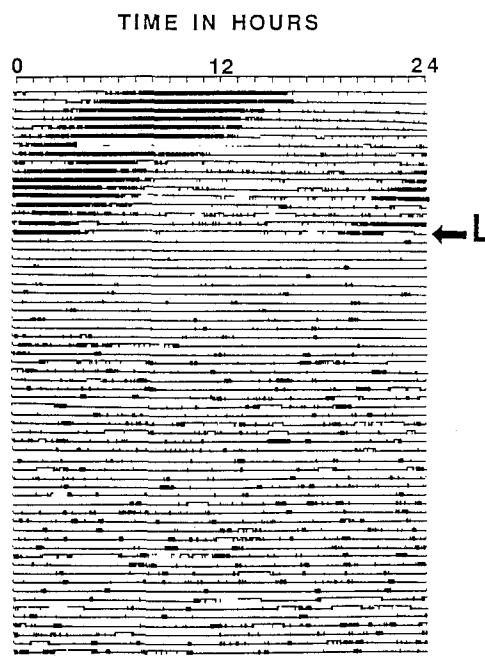


Figure 5. Locomotor activity pattern of a lizard (*Dipsosaurus dorsalis*) held under constant conditions and subjected to a lesion (L) of the suprachiasmatic area of the hypothalamus. The lizard showed a well-defined circadian rhythm of activity until the day of the lesion. Subsequently, the activity became arrhythmic (28, courtesy D. Janik).

shedding of discs from photoreceptor outer segments, visual sensitivity, and NAT activity show daily rhythms and, in some cases, these rhythms have been shown to persist in constant conditions<sup>5–7, 10, 35, 42</sup>. In one study the site of the oscillator driving an ocular rhythm was shown to reside within the eye itself<sup>7</sup>. This study utilized an in vitro eye explant preparation from the African clawed frog, *Xenopus laevis*, and showed that a rhythm in NAT activity could persist in the isolated eye in constant conditions<sup>7</sup>.

Some rather paradoxical effects of blinding on the circadian system of the lizard *S. occidentalis* also suggests that the eyes may play an important role within the lizards' circadian system<sup>55</sup>. Blinding *S. occidentalis* by optic nerve section actually renders them more sensitive to the effects of light since: 1) single light pulses cause a larger shift in the phase of the freerunning activity rhythm in blind lizards than in sighted ones, 2) blind lizards reentrain more rapidly than sighted lizards to a shift in the phase of an entraining LD cycle, and 3) the limits of entrainment are larger in blind lizards than in sighted lizards<sup>55</sup>. One possible model to explain these results would postulate that the eyes can act as oscillators within a multioscillator circadian system. Uncoupling the ocular oscillators from the rest of the system renders the remaining components more sensitive to the phase shifting effects of light.

In some lower vertebrates the eyes are capable of synthesizing melatonin and there is some evidence that the photoreceptors within the eye are the sites of this melatonin

synthesis<sup>7, 29, 41, 42, 66-68</sup>. In some species, such as *Xenopus*, melatonin does appear to play a role in controlling some daily rhythms within the eye itself<sup>42</sup> but it is not currently known if retinally produced melatonin is capable of acting on the circadian system at sites outside the eyes.

#### Circadian organization in lower vertebrates: A model

The results from studies on the circadian systems of a number of species of lower vertebrates can be summarized in the following model (fig. 6). Light and temperature, the primary entraining stimuli for poikilotherms, can act at several different levels of the circadian system. First, both light and temperature can be transduced into a hormonal signal (melatonin) at the level of the pineal. Second, light can act via extraretinal-extrapineal receptors (ERRs) to entrain central oscillators and, since temperature cycles can entrain the activity rhythm of pinealectomized lizards (Underwood, unpubl.), temperature can also directly entrain extrapineal oscillators. Third, light (and possibly temperature) can also act on the circadian system via the eyes.

The pineal, itself, may be autonomously rhythmic and comprised of a population of coupled oscillators. Light and temperature can entrain (and phase) the output of these oscillators (melatonin) so a particular melatonin profile (i.e., phase, amplitude, or duration) reflects the particular environmental stimuli to which the animal is exposed. The pineal melatonin rhythm, in turn, is responsible for entraining circadian clocks located elsewhere (possibly in the SCN) so that these oscillators assume phase-relationships appropriate to those environmental conditions. The coupling strength between the extrapineal oscillators determines the behavior of the system after pinealectomy (i.e. whether splitting, arrhythmicity, or period changes occur). Whether feedback from ex-

trapineal oscillators to the pineal occurs is unknown. Some data suggest that the eyes may also be the loci of circadian oscillators but the importance of these ocular clocks within the animals' total circadian system is unknown.

In some species (e.g., the iguanid lizard *D. dorsalis*) the pineal may play only a minor role within circadian organization and, in these species, the pineal may be unable to sustain a melatonin rhythm in the absence of periodic inputs.

According to the model shown in figure 6 environmental stimuli can act at more than one level on the circadian system. Some as yet poorly understood selective advantages must accrue to such a system. Undoubtedly, aspects of this kind of system must also reflect the organisms' evolutionary history. For example, extrapineal photoreceptors may well have evolved early and then been retained after the evolution of the eyes. Or the pineal may have acted as a primitive photoreceptor with melatonin acting only within the pineal itself. Later, melatonin may have been secreted into the blood and evolved a more 'systemic' role within the animals total circadian 'system'.

Circadian organization in the lower vertebrates clearly involves a number of components including the pineal, the eyes, and the SCN. However, it is becoming increasingly apparent that the relative roles that these sites play between species can vary. This variation can be seen even at the level of the family (e.g. lizards of the family Iguanidae). Little is known about the reasons for this variation but they probably reflect the different selection pressures operating on animals which occupy diverse ecological and temporal niches.

The major weakness of the model depicted in figure 6 arises from the fact that it represents a composite drawn from the results of experiments on a variety of species but, in no case, has the role of all of the elements depicted

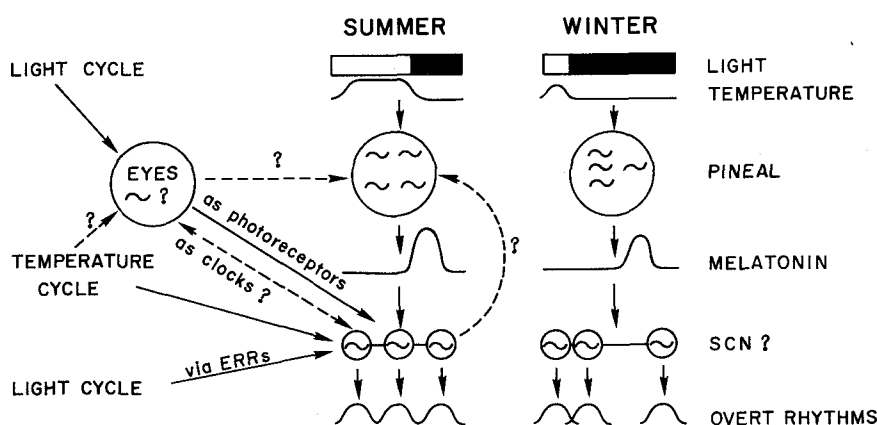


Figure 6. A model for the circadian system of lower vertebrates. Light and temperature conditions (i.e., summer versus winter conditions) are transduced by the (multioscillator) pineal into a melatonin pulse which, in turn, controls both the period and phase of circadian oscillators located elsewhere (i.e., in the SCN?). Other components of the system (shown in the left panel only) include: 1) the eyes which act as photoreceptors and

(possibly) as biological clocks, 2) 'direct' entrainment of extrapineal oscillators by temperature and light (via extraretinal photoreceptors) and, 3) possible links between central oscillators and oscillators in the pineal and eyes. This system allows coordination between internal circadian clocks, and between those clocks and the external world, so that the right events occur at the 'right time of day'.

been thoroughly examined in a single species. Clearly, a major goal of future research should be a comprehensive examination of the roles of the individual elements of the circadian 'system' within individual species while paying close attention to the particular niche which the species occupies. These data would increase our confidence in the model and would yield insights into why different species have opted to emphasize one element over another.

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## Melatonin biosynthesis in the mammalian pineal gland

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**Summary.** Rhythmic production of melatonin by the mammalian pineal occurs in response to noradrenergic stimulation which produces a cascade of biochemical events within the pinealocyte. In the rat, massive changes in NAT activity result from an increase in intracellular c-AMP levels produced by a synergistic interaction whereby an  $\alpha_1$  activation amplifies  $\beta$ -adrenergic stimulation. The intracellular events mediating this effect are described. A major aspect of the temporal control of melatonin production is the programmed down-regulation of responses to noradrenergic stimulation once the initial surge of c-AMP is produced. Noradrenergic activation of the gland also influences other enzymic functions, including tryptophan hydroxylase and HIOMT activities, and produces a dramatic increase in intracellular c-GMP levels. Other neurotransmitters and neuropeptides, e.g. VIP, may also influence pineal function and comparisons are made between the rat, the subject of the bulk of experimental studies, and other species. **Key words.** Melatonin; adrenergic receptors; second messengers; serotonin N-acetyltransferase; hydroxyindole-O-methyltransferase.

In the last few years considerable evidence has accumulated which firmly implicates melatonin produced by the pineal gland as a regulator of the dramatic changes in reproductive function which occur in seasonally breeding mammals<sup>93</sup> (Bartness and Goldman, this issue). Other seasonal changes in physiology are probably also regulated by melatonin (Ebling and Foster, this issue). As day

length changes through the seasons the day/night pattern of melatonin synthesis and secretion is subtly modified. Of the various features of the pattern of melatonin secretion it appears that the duration of the night-time elevation of melatonin is critical<sup>13</sup>. The mechanisms which regulate the seasonal variation in the duration of the melatonin signal are not understood. It seems possible,