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Vertebrate circadian rhythms: Retinal and extraretinal photoreception

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Vertebrate extraretinal photoreception has attracted the interest of biologists for at least a century. Most of the earlier observations dealt with the phototactic and photokinetic behavior of blind fish and amphibians. Although the location of this photoreception was not established it was often assumed that the photoreceptors resided in the skin, a so-called 'dermal light sense'. Interest in extraretinal photoreception has deepened within the last 40 years with the discovery that other important physiological, behavioral, and biochemical events are also controlled, at least partially, by extraretinal photoreceptors[1]. The present discussion will focus on the role of retinal and extraretinal receptors in the entrainment (synchronization) of vertebrate circadian rhythms by daily light-dark (LD) cycles.

All eukaryotic organisms display daily rhythms which persist under constant conditions with periods of approximately, but rarely exactly, 24 hours[2]. Such rhythms have been termed 'circadian' (circa, about, dies, a day) and are driven by an internal 'biological clock'. Among vertebrates literally hundreds of circadian rhythms have been described such as rhythms in enzyme activities, hormone concentrations, DNA and RNA synthesis, electrolyte concentrations in urine and plasma, electrical activity in the brain, and locomotor activity. Locomotor activity is probably the most commonly used assay for the state of an animal's biological clock since it is easy to measure and requires no restraints upon the animal. It has become apparent in recent years that vertebrates are 'multioscillator' in nature; that is, individuals possess more than 1 circadian clock[3-10]. In most cases, however, all of an organism's many overt circadian rhythms exhibit the same frequency and bear fixed phase relationships with one another. Organization of multioscillator systems could be the product of mutual coupling among constituent oscillators so that they all express the same frequency or, alternatively, circadian organization could result from a hierarchical arrangement in which a 'master' circadian pacemaker unilaterally entrains other subordinate (or slave) oscillators[2]. Most likely, vertebrate circadian systems show both mutual and hierarchical organization.

Although details of the sites of circadian pacemakers in vertebrates, and of the photoreceptors mediating entrainment, are far from complete it is clear that the region of the brain adjacent to the third ventricle is of paramount importance. For example, both the retinal, and possibly the extraretinal, photoreceptors mediating entrainment are derived from this area and a pair of nuclei situated at the base of the third ventricle – the suprachiasmatic nuclei – are clearly involved in vertebrate circadian organization. In addition, the pineal organ, which is of major importance in circadian systems of submammalian vertebrates, is also closely related to the third ventricle. Pineal organs are derived embryologically as evaginations of the roof of the diencephalon and, with few exceptions, are ubiquitous in vertebrates. Some lower vertebrates, however, also possess a 2nd component which may arise as an outpouching from the pineal organ or as a separate diverticulum from the diencephalon. This 2nd component is generally termed a parapineal organ and, more specifically, is termed a 'frontal organ' in anuran amphibians or a 'parietal eye' in lizards. Both the pineal and parapineal organs of fish, amphibians and most reptiles are definitely photosensory on both neurophysiological and ultrastructural evidence[11-13]. Although details of ultrastructure and innervation are beyond the scope of the present discussion a number of reviews can be consulted for additional information[11-14]. In general, however, the photoreceptive cells in the pineals of lower vertebrates become degenerate or lost in birds and mammals concomitant with a shift in innervation from a primarily afferent (pinealo-fugal) innervation in lower vertebrates to a primarily efferent (pinealo-pedal) innervation in birds and mammals. All pineal organs show evidence of secretory capabilities[11,14,15]. Much interest has focused on the ability of pineals to synthesize a variety of indoleamines including 5-methoxy N-acetyltryptamine (melatonin). A remarkable feature of vertebrate pineals is the presence of daily rhythms in enzyme activities and biochemical concentrations, including melatonin[15]. Historically it was believed that the terminal enzyme in melatonin synthesis, hydroxyindole-O-methyltransferase (HIOMT) was confined to the pineal, consequently melatonin was considered to be a unique pineal product. More recently a few other tissues have also been shown to have melatonin synthesizing capabilities including the retina and the Harderian gland[16-22].

Fish

The participation of extraretinal photoreceptors (ERRs) in the entrainment of circadian rhythms in fish has been assessed in the eel *Anguilla anguilla*[23], the trout *Salvelinus fontinalis*[24-25], the lake chub *Couesius plumbeus*[26], and the pencil fish *Nannostomus beckfordi anomalus*[27]. In *Anguilla* neither blinding nor blinding combined with pinealectomy abolished entrainment of the circadian activity rhythms to LD 12:12 light cycles[23]. In *Salvelinus* exposed to natural lighting conditions in Sweden, intact fish entrained from mid-August through April but showed freerunning or arrhythmic behavior during the rest of the year[24,25]. Blinded or blinded-pinealectomized trout, however, showed only weak entrainment until December–January after which the activity rhythms were entrained similarly to control fish. In normal lake chub, *C. plumbeus*, entrainment of the activity rhythm to 24-h LD cycles occurred over a wider range of wavelengths (368–742 nm with a maximum sensitivity at 538–568 nm) than in blinded or blinded-pinealectomized fish in which entrainment was restricted to 568–742 nm[26]. The shift in sensitivity toward the longer wavelengths in blinded fish probably reflects the fact that longer wavelengths of light can more readily penetrate tissue and thereby stimulate ERRs in the brain[28]. Also the intensities of light required to entrain blinded or blinded-pinealectomized lake chub were greater than in normal fish. In the pencil fish, *Nannostomus*, a circadian rhythm in color change is present which remains entrainable by light after blinding[27].

The activity rhythms of fish under natural conditions at the arctic circle have significant implications concerning the nature of circadian organization[29-31]. Activity patterns switch from diurnal to crepuscular (during which 2 activity components are clearly present) to nocturnal depending on time of year. These data suggest that 2 oscillators are involved which are only loosely coupled to each other but are locked onto dusk and dawn. This kind of flexibility might allow the fish to exploit seasonally dependent changes in the temporal distribution of food organisms or to undertake migration at a time of day which allows them to escape the attention of predators.

Removal of the pineal organ of several species of fish including the burbot, *Lota lota*, and the lake chub, *C. plumbeus*, has significant effects on the period of the activity rhythm expressed in constant darkness[32,33]. Typically pinealectomy of freerunning fish can cause changes in the period of the rhythm as well as an increased variability in activity onsets. However, pinealectomized fish are still entrainable by LD cycles.

Amphibians

Several laboratories have investigated the role of extraretinal photoreceptors in the entrainment of activity rhythms in amphibians[34]. Both intact and blinded green frogs, *Rana clamitans*, entrain to 24-h LD cycles whereas blinded frogs with the frontal organ removed do not, suggesting that the frontal organ, alone, is capable of mediating extraretinal entrainment[35]. However, blinded newts *Notophthalmus viridescens*, which naturally lack a frontal organ, can still entrain to LD cycles[36]. Although blinded-pinealectomized newts were also tested, the data did not allow definitive conclusions about the role of the pineal due to the small sample size and the 'noisiness' of the data.

There is also ample evidence that extraretinal receptors can mediate compass orientation in amphibians[34]. Many amphibians can steer in a particular direction without the use of landmarks. This kind of orientation involves the use of celestial cues and requires the participation of the circadian clock to compensate for the earth's rotation with respect to such cues. For example, if an amphibian is entrained to an artificial 24-h LD cycle 6 h out of phase with the natural light cycle, upon reexposure to natural days, the animal will orient with a 90° error. The cricket frog, *Acris gryllus*, can orient in a predicted direction to the sun even if the eyes are removed[37]. However, orientational ability is lost in blinded, but not sighted frogs, if an opaque cap is placed over the skull. Similar results were obtained with the tiger salamander, *Ambystoma tigrinum;* eyeless salamanders could orient but eyeless animals with an opaque head covering could not[38]. Blinded-pinealectomized salamanders also failed to orient but salamanders with either the eyes or pineal intact continued to orient in the correct direction[39]. These data suggest that either the eyes or the pineal organ can mediate celestial orientation. Interestingly, tiger salamanders can perceive linearly polarized light and use it for orientation even after blinding[40]. However, insertion of an opaque shield under the skin of the head abolishes orientation to polarized light even in sighted salamanders. The orientational abilities of bullfrog tadpoles, *Rana catesbeiana,* have also been tested after various surgical manipulations: blinded tadpoles, blinded-frontalectomized tadpoles, blinded-pinealectomized tadpoles, and sighted tadpoles with the frontal organ and pineal organ removed could still orient with respect to the sun[41]. If the eyes, frontal organ, and pineal were all removed, however, orientation was abolished suggesting that any one of these organs could act as routes of photoreception.

Administration of daily pulses of exogenous melatonin to tiger salamander larvae at 12.00 h caused the larvae to shift their directional response about 90° clockwise suggesting that the melatonin pulses phase-delayed the salamanders' circadian clock[42].

A recent study has shown that electrophysiological responses to white light can be recorded from the diencephalon and mesencephalon even after removal of the lateral eyes and pineal system in the frog *Rana esculenta*[43]. Typically, sustained multiunit activity occurred in response to a white light stimulus whereas no activity occurred in the dark. There is, however, no direct evidence that these light-sensitive neurons can mediate entrainment of the frog's circadian clock.

Reptiles

Eight species of lizards representing 4 families (Iguanidae, Gekkonidae, Xantusiidae, Lacertidae) have been tested for entrainment to 24-h fluorescent light cycles after blinding[44,45]. All blinded lizards readily entrained to this stimulus even after removal of the pineal system (fig. 1). Shielding the brains of blinded lizards entrained to a dim (0.05 lx) green LD cycle causes them to freerun and blinding lizards entrained to a dim green LD cycle also causes them to freerun[44]. These data show: a) ERRs located in the brain can fully mediate entrainment in lizards, b) the eyes are

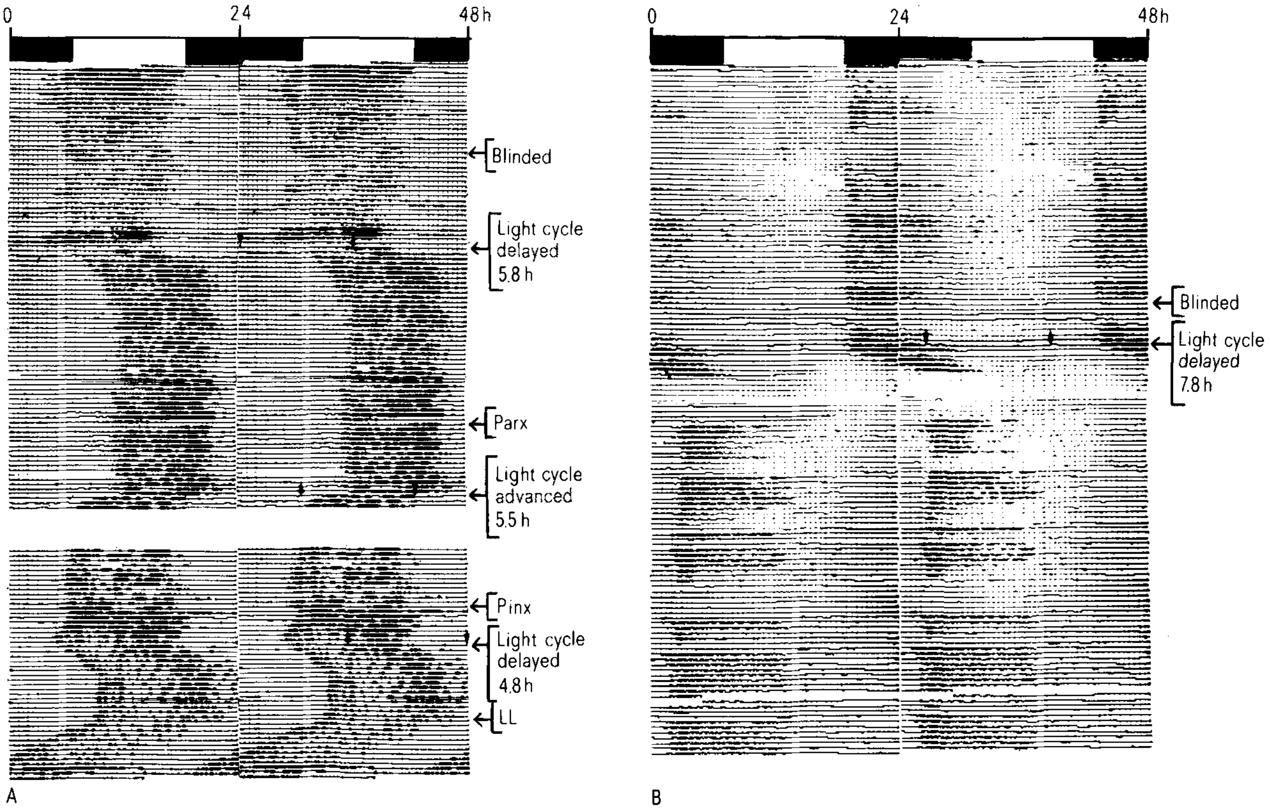

Figure 1. Examples of the entrainment of the activity rhythms of the lizards (A) *Sceloporus olivaceus* and (B) *Coleonyx variegatus* after removal of the lateral eyes and pineal system. The initial LD 12:12 fluorescent light cycle is diagrammed at the top of the record. The records are presented in duplicate to aid in interpretation. The time of onset and offset of phase-shifted light cycles are shown by upward and downward pointing arrows respectively. 'Parx', parietal eye removed; 'Pinx', pineal organ removed; 'LL', continuous illumination. The nocturnal *Coleonyx* was pinealectomized before the beginning of the record. A parietal eye is absent in *Coleonyx*.

also involved but whether they can support entrainment in the absence of ERRs is unknown and, c) the pineal system (pineal-parietal eye) is not necessary for entrainment of blinded lizards but the possibility that it can act as an alternative route of photoreception is still a viable possibility. Interestingly, blinding the lizard *Sceloporus olivaceus* while freerunning in continuous illumination causes a shortening of the period of the rhythm or arrhythmicity - effects which are not explicable on the basis that blinding has merely removed a photoreceptive input to the circadian clock[45].

Phototactic responses of the alligator *Alligator mississippiensis* show circadian rhythmicity in both normal and opaque eye-covered individuals[46,47]. Alligators are one of the few vertebrate species which appear to lack a recognizable pineal organ. The threshold energy required to obtain a positive phototactic response was directly dependent on temperature in intact and in eye-capped alligators but in alligators receiving only retinal inputs (head capped) this temperature dependence was absent[46].

Although entrainment of blinded lizards persists after removal of the pineal system, pinealectomy does have significant effects on freerunning lizards (fig. 2)[48,49]. In *S. olivaceus*[48] pinealectomy can induce splitting of activity rhythms into separate circadian components, changes in the period of the rhythm or arrhythmicity (50% of cases) whereas in *S. occidentalis*[49] arrhythmicity after pinealectomy is more rare (10% of cases) and period changes are usually seen. The effects of pinealectomy in lizards are compatible with at least 2 general hypotheses: 1. the pineal organ controls the frequencies of the circadian oscillators comprising the lizard multioscillator system by acting as a master circadian pacemaker or 2. the pineal is not the locus of a master circadian pacemaker but acts as a coupling device among circadian oscillators located elsewhere. The various changes seen after pinealectomy in lizards would not reflect qualitative differences in circadian organization but would reflect quantitative differences in the integrity of the system remaining after pinealectomy. Changes in the period of the rhythm or arrhythmicity, for example, would occur if coupling between oscillators was weakened or abolished or if the frequencies of subordinate oscillators was altered after removal of a pacemaker.

Circadian rhythms in indoleamine synthesis have been demonstrated in the pineal and retinas of reptiles[20,50]. Continuous exogenous administration of melatonin via silastic implants causes a lengthening in the freerunning activity rhythm in intact, pinealectomized, blinded or blinded-pinealectomized *S. occidentalis*[49,51].

Birds

The locomotor activity rhythm of blinded house sparrows, *Passer domesticus*, can be entrained by light cycles even at low intensities and, further, pinealectomy does not abolish entrainment in blinded *Passer*[52,53]. The brain is the locus of this extraretinal photoreception. Retinal photoreceptors are also involved in entrainment since the light threshold for entrainment is lower in sighted than in blinded *Passer*[54]. The arrhythmicity induced by exposure of house sparrows to sufficient intensities of continuous illumination also depends on retinal photoreception[55].

Robust rhythms of indoleamine synthesis are present in the pineal and retina of birds (fig. 3)[4-6,17,22]. The avian pineal can possess cells which have photoreceptive characteristics although the outer segments are degenerate in appearance. Although electrophysiological recordings have failed to prove a direct photosensory role for the avian pineal[56,57], biochemical activities within the pineal can be altered by photoperiod in organ cultured pineals showing a direct photoreceptive capacity by avian pineals[4-6,58]. Significantly, circadian rhythms in melatonin and N-acetyltransferase (NAT) activity persist in organ cultured pineals or even within a dispersed cell culture (NAT rhythm) and these rhythms are entrainable by LD cycles[4-6].

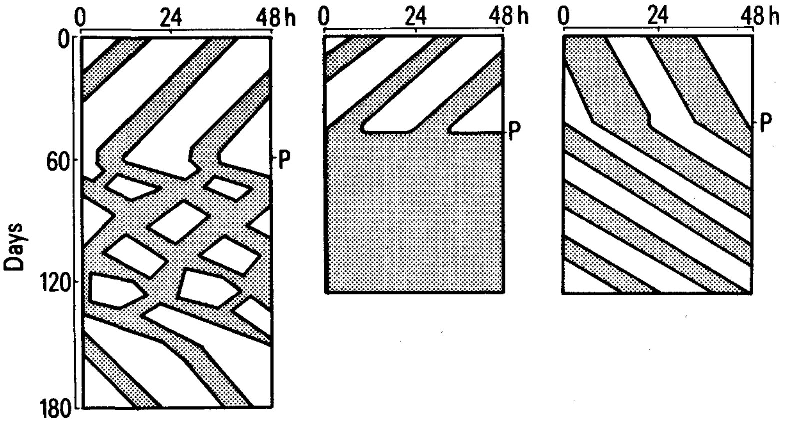

Figure 2. Types of response of lizards to pinealectomy (P) while freerunning in constant conditions. The record on the left shows a splitting of the activity rhythm into 2 circadian components after pinealectomy, the middle figure shows arrhythmicity and the figure on the right shows a change in the period of the freerunning rhythm.

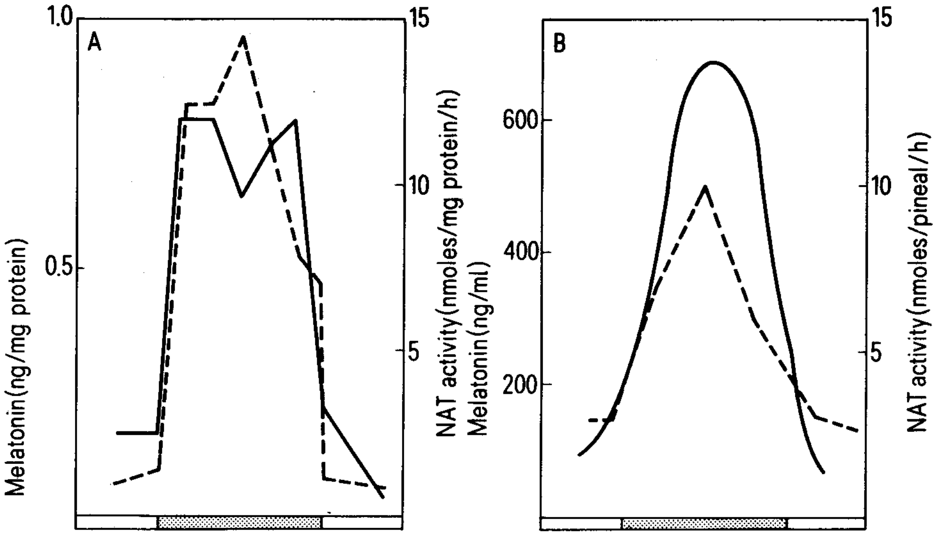

Figure 3. Rhythms in N-acetyltransferase activity (dashed lines) and melatonin (solid lines) in the chick retina in vivo (A) and pineal in vitro (B)[4,6,17]. NAT has been implicated as the rate limiting enzyme in melatonin synthesis.

Pinealectomy affects the freerunning activity rhythms of some species of birds[59-62]. For example, pinealectomy of house sparrows freerunning in continuous darkness (DD) produces arrhythmicity whereas implantation of a pineal into the eye of such an arrhythmic bird reintroduces rhythmicity[60-62]. Significantly, the induced rhythms shows the same phase as that shown by the donor bird. Exogenous melatonin administration via silastic capsules can induce a shortening of the period of activity rhythms or arrhythmicity in house sparrows[63] and daily melatonin injections can entrain the activity rhythm of pinealectomized starlings *Sturnus vulgaris*[64]. Figure 4 presents the current model for the circadian system of birds such as the house sparrow or the starling.

Mammals

Extraretinal photoreception is absent in adult mammals[68-74]. After blinding circadian rhythms in blood eosinophil levels (rats)[69], pineal NAT activity (rats)[68], and locomotor activity (mice, rats, monkeys)[70-74] are no longer entrainable by LD cycles. Entrainment fails even if the amount of light reaching the brain is greatly increased by surgically creating 'windows' over the brain[75].

Lesions of the suprachiasmatic nuclei (SCN) of mammals abolish a variety of circadian rhythms including those of locomotor activity, blood corticosterone levels, and pineal NAT activity[76,77]. Significantly, a circadian rhythm in electrical activity of the SCN can still be recorded after the SCN has been isolated from the rest of the brain by knife cuts[3]. These data suggest that the SCN is the locus of a circadian clock(s) which drives all of the animal's overt circadian rhythms. A direct retino-hypothalamic pathway is the route by which lighting information reaches the SCN (fig. 5)[76,77]. The mammalian pineal deprived of sympathetic innervation does not exhibit rhythmicity and, in contrast to the other vertebrate classes, only slight effects of pinealectomy have been noted on mammalian activity rhythms[78-80]. In the mammal pineal rhythmicity is driven by the SCN. The SCN communicates with the pineal by a neural pathway that includes the medial forebrain bundle, preganglionic fibers from the cervical spinal cord to the superior cervical ganglia, and post-ganglionic sympathetic fi-

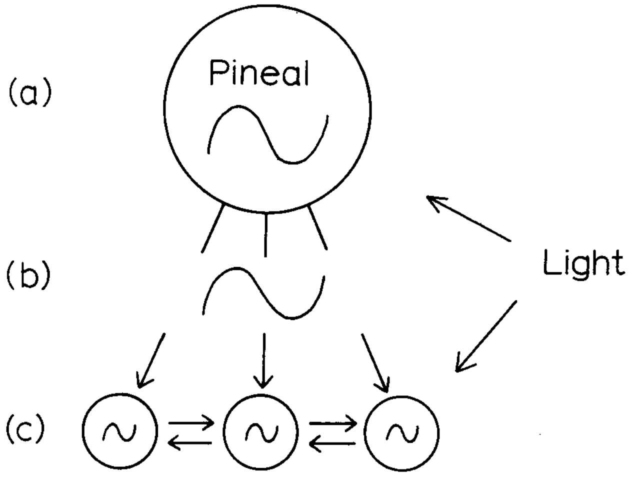

Figure 4. A model for the circadian system of birds[59]. Elements include (a) a circadian pacemaker in the pineal which controls the frequencies of subordinate oscillators (c) located outside the pineal possibly via the rhythmic release of a hormone (i.e., melatonin) (b). The subordinate oscillators may themselves show mutual coupling. (The fact that lesions of the suprachiasmatic nuclei (SCN) disrupt circadian rhythms in house sparrows and Java sparrows *(Padda oryzivora)* makes the SCN a possible candidate for the location of the subordinate oscillators or for the site of coupling)[61,65]. Light may affect pineal activity directly[4-6,58], via the retina[11], or possibly via ERRs[66]. Light can also entrain the subordinate oscillators via ERRs[53] and possibly via the retina[67]. Since pinealectomy has been reported to be without effect in gallinaceous birds[61], the more central circadian oscillators may play the dominant role in some avian species.

bers from the superior cervical ganglia to the pineal (fig. 5)[77]. A small but growing body of evidence suggests that additional neural routes to the mammalian pineal may also exist[81-83].

Continuous light (LL) can cause degeneration of photoreceptor outer segments[84-87]. In adult mammals certain light-dependent reactions remain intact after LL 'blinding' such as light-dark discrimination[86] or entrainment of the plasma corticosterone rhythm[87], suggesting that the light sensitive cells within the eye mediating these reactions are not the classical photoreceptive cells. This possibility, however, has become somewhat less tenable with the suggestion that these light responses may be mediated by small populations of photoreceptors which are resistant to LL degeneration[84,85].

In juvenile rats extraretinal photoreception has been implicated in the control of daily rhythms in pineal serotonin and HIOMT activity[88-92]. In newborn rats up to 20 days of age a pineal serotonin rhythm persists under LD cycles after sympathetic innervation has been abolished[88-91]. The pineal serotonin rhythm observed in the denervated pineal of neonatal rats appears to be exogenously driven by light and is not a true circadian rhythm since it does not persist under constant conditions[88]. An HIOMT rhythm also persists under LD cycles in blinded 12-day-old rats[92]. The Harderian gland has been implicated as the ERR involved since its removal abolishes the effects of light on serotonin or HIOMT in blinded juvenile rats[90,92]. If the Harderian gland is involved it must be connected to the pineal via hormonal links since sympathetic denervation of the juvenile rat pineal does not abolish the effects of light on pineal serotonin[88,89]. However, a direct photosensitivity of the juvenile rat pineal is possible and is consistent with the photoreceptive appearance of developing pinealocytes in neonatal rats[93]. In neonatal rats between the ages of 4 and 12 days the pinealocytes become elongated and polarized and develop cell processes from which extend cilia with a 9+0 arrangement of microtubules. Lamellated and vesicular membranes are seen at the tips of the cilia. These morphological features are transient and are not seen in rats older than 17 days. Complete pineal innervation only occurs after 2 weeks of age[94] and the SCN appears to become completely functional by day 21[95]. Apparently extraretinal photosensitivity is lost after the pineal comes under the neural control of central circadian clocks.

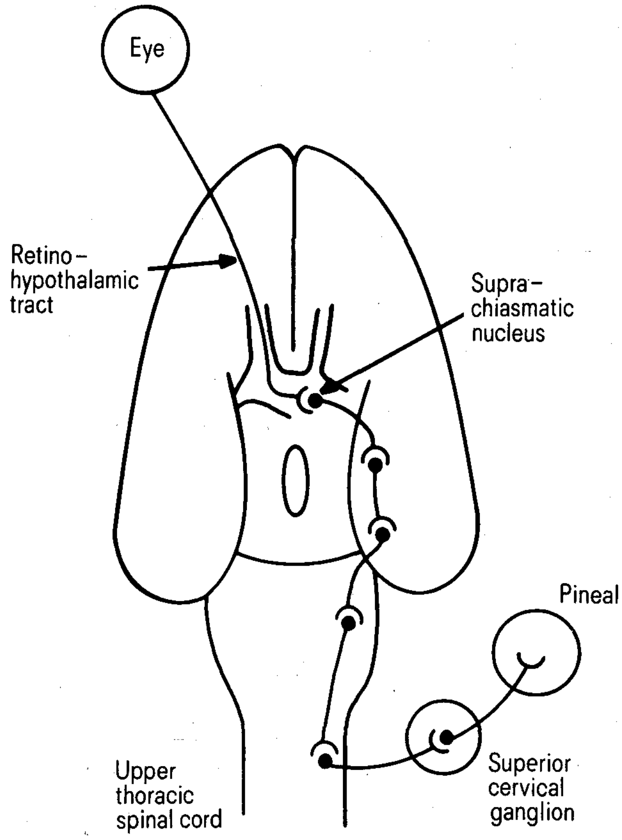

Figure 5. Pathway of light to the SCN and the pineal in adult mammals.

Summary: the eyes

Clearly the eyes, in addition to ERRs have a photoreceptive input into the circadian clock of submammalian vertebrates. 1. Blinded animals tend to have a higher light threshold for entrainment than sighted animals[26,44,54]. 2. Entrainment persists in sighted animals after blocking light penetration to ERRs[37,38,54]. 3. The ability of animals to entrain to LD cycles can be manipulated by altering the relative amounts of light reaching the eyes and extraretinal receptors[54] and, 4. arrhythmicity induced by continuous illumination may be a function of retinal photoreception[55]. In adult mammals the eyes are the only route for the perception of entraining light cycles[70-74].

A small but growing body of evidence also suggests that, in some species, the eyes may play an additional role in circadian organization. Recent studies have shown that the shedding of discs from the outer segments of the photoreceptors of all classes of vertebrates show a daily rhythm: a burst of membrane shedding occurs at the same time each day[96-99]. In some species this shedding event persists under constant conditions[96-98]. In the rat this rhythm seems to be driven by an oscillator(s) residing within the eye; disc shedding persists after sectioning of the optic nerve but requires an intact nerve for entrainment by light[98]. These results suggest that a circadian oscillator resides in each eye and this oscillator is normally entrained by an oscillator(s) in the central nervous system. Intraocular rhythms in indoleamine metabolism are probably also ubiquitous among vertebrates although the locus of oscillator(s) controlling these rhythms have yet to be established[17,18,22]. However, pinealectomy does not affect the NAT rhythm in the eye (birds)[17] or the disc shedding rhythm (in rats)[99]. The possibility that indoleamine rhythms regulate other ocular rhythms (such as disc shedding) is an attractive possibility. The eyes may also release melatonin into the blood which may affect circadian oscillators residing elsewhere. Clearly, the vertebrate eye may have a variety of neuroendocrine functions which are as yet unappreciated.

Summary: ERRs

Both the pineal and the SCN are elements of the vertebrate multioscillator system although the relative importance of these 2 areas probably varies between, and possibly within, the different vertebrate classes. Extraretinal photoreception is a universal feature of submammalian vertebrates, and possibly of neonatal mammals, but is absent in adult mammals. Although the pineal systems of sumammalian vertebrates are photosensitive, the pineal system has been directly implicated as an extraocular site for the perception of entraining light cycles only in amphibians. In all other submammalian vertebrates *extraretinal entrainment* can occur in the absence of the pineal system although it is certainly conceivable that the pineal system may act as an alternate route of photoreception. These extraretinal-extrapineal receptors are located within the brain but the exact location(s) of these receptors within the brain is unknown. The hypothalamus would be likely area for this extraretinal photoreception, however, for several reasons: 1. Neurophysiological studies have identified light sensitive neurons in the frog's hypothalamus[43]. 2. The avian hypothalamus is a site of photoperiodic photoreception[100-103]. 3. The only other light sensitive structures known in vertebrates - the pineal system and the lateral eyes - are all derived embryologically from the hypothalamus. 4. The hypothalamus appears to be the site of a circadian clock and there may be advantages in having the photoreceptors and the clock anatomically close to one another. These considerations, of course, do not exclude the possibility that other brain areas may be involved as well.

The reason behind the loss of extraretinal photoreception in mammals is uncertain. The shift to exclusive retinal photoreception in mammals may have been dictated by the extensive reorganization that occurred during the evolution of the mammalian brain. Or, perhaps, the increased size of the mammalian skull and overlying tissue made direct photoreception difficult and necessitated a shift to retinal photoreception. The persistence of extraretinal photoreceptors in submammalian vertebrates, however, underscores their importance in the sensory repertoire of vertebrates.

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Brain photoreceptors for the photo-induced testicular response in birds*

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Gonadal function in many birds is stimulated by visible radiations, and daylength constitutes a primary source of information for controlling the reproductive cycle; this was first demonstrated by Rowan[1]. Testicular development depends upon the gonadotropic activity of the hypothalamic-adenohypophyseal system (review in Baylé[2]), and two hypothalamic regions in particular participate in the neuroendocrine control of gonadotropic secretion: the infundibular complex and the anterior-preoptic area. The mechanisms by which birds perceive light in order to induce gonadotropin release are discussed in this report.

Originally, it was thought[3] that the photoreceptors for photoperiodic gonadal responses in birds were located in the testes themselves and in the skin of the foot. However, artifical lighting of the whole body, except for the head which was covered by a black hood, did not induce the photosexual reflex in ducks[4] and sparrows[5,6]. Therefore, it was assumed that the eyes contain the photoreceptors, and Benoit concluded from a long series of experiments that the eyes do indeed contribute to the stimulation of testicular growth by light. When, for example, the retinal photoreceptors were isolated by slats of opaque rub-