Extraretinal photoreception in entrainment and photoperiodism in invertebrates*

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The regular fluctuation in light intensity that is the most prominent physical correlate of the solar day provides reliable information about both the time of day and the time of year for many organisms. In the past two decades a number of investigations have been devoted to the identification of photoreceptive pathways for the perception of these changes in light intensity, and to the elucidation of their role in the appropriate phasing of daily and seasonal variations in physiological, behavioral and metabolic processes.

A curious fact, whose functional significance is not well understood has emerged from these studies. Even in groups such as the molluscs and arthropods that have well developed 'organized' photoreceptive structures many (though not all) species utilize extraretinal photoreceptors in the transduction of the temporal information provided by the environmental light cycle. Thus extraretinal photoreception plays a central role in the entrainment of circadian oscillations and in photoperiodic time measurement in many invertebrates (as well as vertebrates – see Underwood and Gross, this volume). In fact, this is the most clearly demonstrated and prevalent functional role for extraretinal photoreception in several major invertebrate groups.

This review is undertaken with 2 goals in mind. The first is to briefly summarize the available information on the function, location and spectral sensitivity of photoreceptors involved in the entrainment of circadian oscillations and in photoperiodic time measurement in invertebrates, particularly the molluscs, crustaceans, and insects. The second is to attempt to identify the generalizations that can be drawn from this work and to point out some of the more pressing questions that remain in our effort to understand the functional significance of this aspect of extraretinal photoreception.

Entrainment of circadian rhythms

Efforts to localize photoreceptors responsible for the entrainment of circadian rhythms in invertebrates have produced a diversity of results. In the molluscs, for example, current evidence suggests both retinal and extraretinal photoreception is involved, while in crustaceans (crayfish) and many insects extraretinal photoreception appears to be the primary, and in some cases only, sufficient pathway. In other insects and probably in scorpions the photoreceptors for entrainment reside solely in the compound eyes (though there are often other data that indicate the presence of extraretinal photoreceptors in these animals).

The clearest demonstration of extraretinal entrainment of circadian rhythms in molluscs comes from recent studies on the locomotor activity rhythm of the garden slug *(Limax maximus* and *Limax flavus)*. Following surgical removal of the optic tentacles the activity rhythm could be synchronized by light cycles; and on subsequent release into constant darkness a freerunning rhythm persisted that was phased by the prior light cycle[1]. Notably, eye removal was not without effect on entrainment. Eye removal led to an increase in the steady state phase angle difference between activity onset and the light to dark transition, and significantly altered the action spectrum for entrainment. While intact animals could readily be entrained by low intensity (< 1 lx) cycles of red light (> 600 nm) eyeless animals freeran in red light cycles even at substantially higher intensities (> 100 lx). The results implicate both ocular and extraocular photoreception in the entrainment pathway[1].

Evidence for the participation of ocular and extraocular photoreceptors in entrainment has also been obtained in the marine gastropod *Aplysia californica*. The isolated eye of *Aplysia* exhibits a circadian rhythm in spontaneous neural activity recorded in vitro from the optic nerve[2] that can be entrained by light cycles presented in vitro[3]. Thus ocular photoreceptors are sufficient for entrainment to white light. On the other hand, entrainment to cycles of moderate intensity red light (> 580 nm) may involve extraocular pathways. When animals with 1 optic nerve cut were placed in cycles of red light only the eye with the intact optic nerve became entrained while the neurally isolated eye appeared to freerun[4]. One explanation of this result is that a red sensitive extraocular photoreceptor is sufficient for entrainment of the rhythm in the eye and that its effect is mediated by efferent activity in the optic nerve. This interpretation is supported by the observation that a diurnal rhythm in locomotor activity can be synchronized by red light cycles after eye removal[5] – clearly the postulated extraocular photoreceptors exist. There are, however, alternative explanations which have not been ruled out that invoke a facilitative rather than photoreceptive role for the efferent activity[4,6].

Aplysia also exhibit a circadian rhythm of locomotor activity. Eye removal severely disrupted the rhythm in constant conditions[5,7,8] however, a diurnal rhythm of activity of eyeless animals could still be driven by a light cycle[5,9]. Thus extraocular photoreceptors are involved in the control of locomotor behavior. Similar results have recently been obtained in another marine gastropod, *Bursatella leachi plei*[10].

The anatomical location of these extraocular photoreceptors is uncertain although there is a wealth of possibilities. Several photosensitive cells have been found in the central nervous systems of molluscs (see Andresen and Brown, this volume) and photosensitivity of the anterior tentacles[11] and rhinophores[12] of *Aplysia* has been reported.

Extraretinal photoreception has also been shown to be involved in the entrainment of circadian rhythmicity in the crayfish, *Procambarus clarkii*. Surgical removal of the compound eyes and most distal neuropile of the optic lobe (the lamina ganglionaris) had no effect on the synchronization of the rhythms of locomotor activity[13] (fig. 1) and heart rate[14]. Furthermore, it has been shown that the circadian rhythm in the amplitude of the electroretinogram (ERG) could be entrained by an extraretinal pathway[15]. In these experiments fiber optics were utilized to present 'localized' light cycles to the compound eye and the brain (supraesophageal ganglion). When one light cycle was presented to the eye and another, 180° out of phase with the first, was presented to the brain the ERG rhythm was invariably entrained by the brain light cycle; and when the animal subsequently placed in constant conditions the rhythm freeran with appropriate phase.

The possibility that the caudal photoreceptor, a well known light sensitive neuron in the 6th abdominal ganglion of crayfish might be involved in entrainment was also investigated. Section of the ventral nerve cord anterior to the 6th abdominal ganglion had no effect on entrainment of either the activity rhythm[13,16], or ERG rhythm[17], nor did complete extirpation of the ganglion affect entrainment of activity. The results suggest entrainment is mediated by an extraretinal-extracaudal photoreceptor that is probably located in the brain.

Attempts have been made to identify photoreceptors for entrainment in several insects. Evidence for the involvement of extraretinal photoreception has been obtained in *Drosophila*, *Carausius*, *Ephippiger*, *Chortippus*, and 3 species of silkmoth.

In the silkmoths *Antheraea pernyi* and *Hyalophora cecropia* the circadian oscillator that controls the time of adult emergence is located in the brain and its effects are mediated via a hormone produced in the neurosecretory cells of the brain. In animals in which the brain is neurally isolated or transplanted from the head to the abdomen the circadian rhythm in eclosion persists and can be entrained by light[18,19]. Truman[19] demonstrated that the photoreceptor for entrainment was located in the brain. The experiment is illustrated in figure 2. Brains were removed from a population of pupae and were either replaced in the head region or transplanted to the abdomen. The pupae were then placed in holes in a partition which separated 2 chambers in which there were 2 light cycles 180° out of phase. Animals that had brains replaced in the head region entrained to the light cycle to which the anterior end of the pupae was exposed. Those in which brains had been transplanted to the abdomen were entrained by the light cycle presented to the posterior end. The results provided a clear demonstration that the photoreceptor resides in the brain. Entrainment of flight activity rhythms in adult silkmoths has also been shown to involve extraretinal photoreceptors that are probably located in the brain[20].

In *Drosophila melanogaster* the eclosion rhythm of the eyeless (and ocelliless) mutant, sine oculis, can be readily entrained by light implicating extraretinal photoreception[21,22]. Evidence from experiments with local illumination suggested the photoreceptor is at the anterior end of the pupae[22,23], and it seems likely

Light cycle delayed

Removed ommatidia, lamina ganglionaris

Cut 5-6 connective

Light cycle advanced

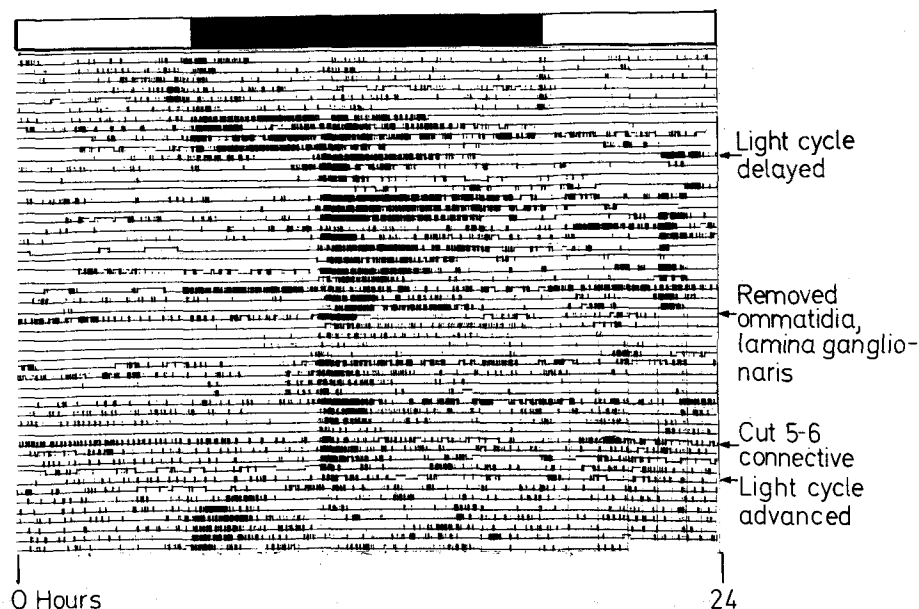0 Hours   24

Figure 1. Activity record of a crayfish in a light cycle of 12 h light and 12 h dark illustrated by the bar at the top of the record. There are 2 peaks of activity; one, an endogenous (circadian) peak associated with the onset of darkness, and a 2nd, exogenously driven peak synchronized to the onset of light. Both peaks followed the 4-h delay in the light cycle. Removal of the ommatidia and first optic neuropile abolished the activity associated with light-on. The light-off peak was retained and entrained to a phase advance in the light cycle even after the caudal photoreceptor was surgically isolated[13].

that, as in the silkmoth, the receptor is located in the brain.

In contrast to the situation in molluscs experiments with both silkmoths and *Drosophila* suggest that the eyes are not only unnecessary, but they are not sufficient for entrainment. In intact silkmoths covering the entire head with opaque wax except for the compound eyes frequently abolished entrainment[20]. In the white-eyed mutant of *D. pseudoobscura* the photoreceptors of the compound eyes are 2 log units more sensitive to light because of a loss of screening pigment, but there was no increase in the light sensitivity of the circadian rhythm[22]. Furthermore, while *D. melanogaster* raised on a carotenoid-free medium exhibited a 1000-fold drop in ERG amplitude, the sensitivity to a phase shifting stimulus was unaffected[24].

Evidence for extraretinally mediated entrainment has also been obtained from surgical ablation experiments in several other insects. Rhythms of oviposition in *Chorthippus curtippenis*[25] and *Carausius morosus*[26] and of stridulation in *Ephippiger ephippiger* and *E. bitterrensis*[27] could still be driven by light following surgical removal of the compound eyes and/or ocelli.

Another interesting example of the involvement of extraretinal photoreception in insect circadian rhythms has been described by Neville[28]. In many insects the formation of the endocuticle occurs as a daily deposition of alternately lamellate and non-lamellate chitin. The rhythm of deposition persists in constant darkness but is abolished by constant light. In locusts *(Schistocerca gregaria* and *Locusta migratoria)* the effect of constant light persisted after ablation of the compound eyes and ocelli, and the results of opaquing experiments indicated the site of photoreception was within the epidermal cells[28].

While extraretinal receptors appear to be common in entrainment in arthropods in some insect species and in scorpions the compound eyes have been shown to be the primary pathway. In both the cockroach[29-31] and the cricket[32] ablation studies have provided un-

equivocal evidence that the compound eyes are the sole photoreceptors for entrainment. And in the beetle, *Blaps gigas*[33] and the scorpion, *Androctonus australis*[34,35] illumination of the eyes was sufficient for entrainment of the ERG rhythms. In the latter case the residual uncertainty that the light was penetrating to extraretinal elements appears to have been ruled out. When the illuminated eye was covered the rhythm began to freerun even though the prosoma was still illuminated.

In summary, efforts to identify the photoreceptors by which light information reaches circadian oscillators in invertebrates have provided us with a rather diverse set of results from which it is difficult to draw firm generalizations. Extraretinal pathways are prevalent, but in molluscs and some arthropods retinal pathways are either involved as alternate or sole sources of photic information for the circadian system.

It is also important to note that even in organisms where the eyes apparently do not participate in entrainment retinal photoreceptors may mediate changes in the pattern of rhythmicity evoked directly by the transition from darkness to light and vice versa as has been demonstrated in the silkmoth[19,20] and crayfish (fig. 1)[13]. In other systems (e.g. *Aplysia*) the converse may ultimately prove to be the case with retinal photoreceptors being predominant in oscillator entrainment and extraretinal receptors acting via pathways which control the pattern of rhythmicity.

Photoperiodic photoreception

In the slug, *Limax maximus*, sexual maturation of male reproductive organs is under photoperiodic control. Enlargement of the gonads is promoted by exposure to long days (16 h light, 8 h dark) and prevented when animals are maintained on short days (8 h light, 16 h dark)[36]. Attempts to localize the photoreceptor involved produced results that paralleled those from investigations of entrainment of the activity rhythm (see above). Discrimination between long and short days of white light was unimpaired by removal of the optic tentacles suggesting extraocular photoreceptors are sufficient. On the other hand, discrimination betwen long and short cycles of red light (> 600 nm) required the optic tentacles be intact[37]. Thus both extraocular and ocular photoreceptors participate in the photoperiodic response.

In insects there are several cases where photoperiodic responses are mediated by extraretinal photoreceptors. In the 2 most thoroughly investigated species; the aphid, *Megoura viciae*, and the silkmoth, *Antheraea pernyi*, the site of photoreception has been localized to the brain.

In *Megoura* the development of female offspring into sexual, egg laying morphs vs asexual, parthenogenetic morphs is determined by the length of the photoperiod to which the mother is exposed. Lees[38] found
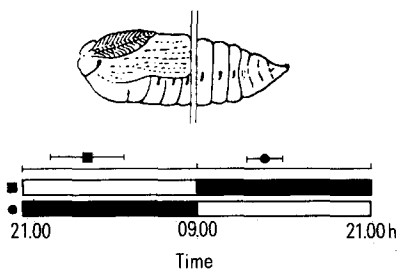
Figure 2. Time of eclosion (mean and SD) of 2 groups of *H. cecropia* which differed in the site of brain implantation. The anterior end of each pupae was exposed to light from 21.00 h to 09.00 h (top bar) and the posterior half was exposed from 09.00 h to 21.00 h (lower bar). ■, Brain implanted into head; ●, brain implanted into abdomen. Moths entrained to the light cycle to which the brain was exposed[19].

that cauterization of the compound eyes did not affect the photoperiodic response indicating extraretinal photoreceptors were involved. Further localization was carried out with fine light guides which were used to extend a short day exposure of the whole aphid into a long day with localized supplemental illumination (fig.3). The results suggested the photoreceptor was in the head region and probably in the protocerebrum. A subsequent study involving small brain lesions provided evidence that was consistent with this view[39].

A brain centered photoreceptor has also been implicated in the photoperiodically controlled termination of diapause in *A.pernyi*. When the anterior and posterior ends of intact pupae were exposed to different photoperiods the maintenance or termination of diapause depended on the photoperiod to which the anterior end was exposed. When the brain was transplanted to the abdomen the ability to discriminate between long and short days was retained but the site of photosensitivity had been transferred to the abdomen[40]. The results are similar to those obtained in studies on entrainment of the eclosion rhythm (see above) and indicate that the locus of the photoreceptor is in the brain. Similar transplantation experiments have implicated the brain as the photosensitive pathway for photoperiodically controlled initiation of diapause in the cabbage butterfly, *Pieris brassicae*[41,42].

Studies on 2 species of beetles have produced contrasting results. In the Colorado potato-beetle, *Leptinotarsa decemlineata* photoperiodically controlled reproductive diapause in females did not require the compound eyes[43]. In contrast, reproductive diapause in male *Pterostichus nigrita*, appears to involve retinal photoreception. Animals in which the compound eyes were ablated responded as if they were in constant darkness regardless of photoperiod[44].

Although the data are more limited, the results of efforts to localize photoreceptors involved in photoperiodic time measurement seem to parallel those obtained in studies on entrainment pathways. Depending on the species retinal, extraretinal, or both types of photoreceptors may be utilized.

Action spectra and sensitivity

Two other aspects of extraretinal photoreception have been investigated in a few invertebrate species – action spectra and sensitivity. There are 3 cases in insects in which relatively complete action spectra have been obtained where photoreception for entrainment *(Drosophila)* or the photoperiodic response *(Megoura, Antheraea)* has been shown to be predominantly mediated by extraretinal pathways.

In *Drosophila pseudoobscura* the effectiveness of 15-min pulses of various wavelengths of light from 375 to 600 nm in evoking either a delay or advance phase shift has been investigated. Both action spectra showed a broad region of sensitivity from 375 to 500 nm with the most effective wavelength being between 420 and 480 nm. Above 500 nm sensitivity fell off sharply[45]. A more detailed spectrum for delay phase shifts (fig.4) was subsequently obtained[46]. The data revealed a peak sensitivity at 457 nm with lower maxima at 473, 435, 407, and 375 nm. These results suggested that the photoreceptor pigment was a flavin or flavoprotein[46], a view consistent with the observa-
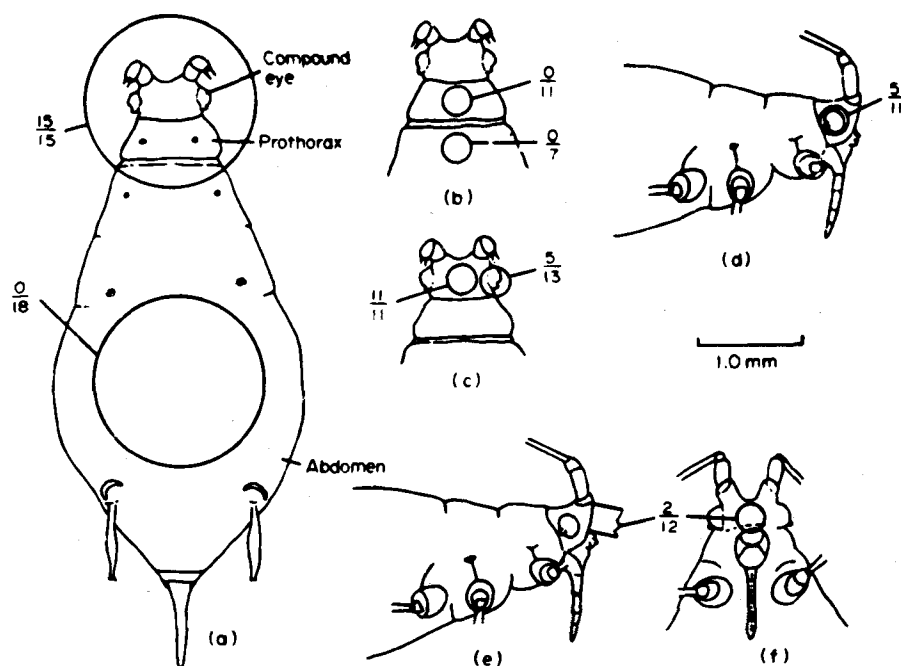

Figure 3. Results obtained from selectively illuminating various regions of *Megoura viciae*. Circles show the area of illumination. The denominator in each fraction indicates the number of aphids tested and the numerator gives the number of positive responses[38].

tion that *Drosophila* raised on a carotenoid free medium exhibited no change in sensitivity to a phase shifting stimulus[24].

In the aphid, *Megoura viciae,* results of action spectrum studies on the photoperiodic response suggested the likely involvement of 2 pigments. Aphids maintained in a light cycle consisting of 13.5 h of light and 10.5 h of dark exhibit a 'short day' response. This response can be reversed by exposing the animals to a brief (0.5–1.0 h) pulse of light at 1 or 2 times during the dark period. The 1st of these light sensitive points, which occurs between 0–3 h after the onset of darkness, exhibited a peak sensitivity at 450–470 nm and fall off rapidly above 475 nm and below 450 nm[47]. The 2nd light sensitive period, which occurs several h later (6–10.5 h after onset of darkness), was also found to be maximally sensitive at 450–470 nm, but its sensitivity extended above 550 nm and down to 400 nm[47].

In *A. pernyi* the action spectrum for diapause termination was measured by extending a 10-h (short) day with an additional 6 h of monochromatic light[48]. The most effective wavelengths were between 400 and 500 nm with sensitivity declining steadily between 500 and 600 nm.

This general trend of peak sensitivity of extraretinal receptors to blue light and relative insensitivity to red light has also been described in *Pieris brassicae*[42] and in the slug *Limax* (see above). Diapause termination in the coddling moth, *Laspeyresia pomonella*[48] and entrainment in *Pectinophora gossypiella*[49], also follow this trend although in the latter two cases extraretinal photoreception in the adult has not been demonstrat-

ed. It is interesting to note that in *Pectinophora* the photoperiodic response is sensitive to red light[50] suggesting the possibility different or additional photoreceptors may be involved in photoperiodic time measurement.

The data on action spectra in insects have also been used to obtain a quantitative estimate of the sensitivity of extraretinal photoreceptors[51]. The results indicated the photoreceptors are quite sensitive and compare favorably with, for example, the phytochrome system in plants. Standard phase shifting or photoperiodic responses are obtained with incident energies on the order of $10^{-1}–1 \text{ J} \cdot \text{m}^{-2}$.

Conclusions

It is clear from the results summarized here that extraretinal photoreception is a pervasive feature of temporal organization in invertebrates. The involvement of extraretinal receptors in entrainment of circadian oscillations has been documented in molluscs, insects and crustaceans; and in several insects and a mollusc extraretinal photoreception has been implicated in photoperiodic time measurement. Yet beyond this broad statement it is difficult to formulate generalizations about either the distribution or functional significance of extraretinal photoreception. 2 factors seem to contribute heavily to this problem. 1st, especially outside the insects, only a very limited number of organisms have been investigated. This is particularly problematical in view of the diversity of results that have been obtained in those species that have been examined. In some the photoreceptors involved in entrainment or photoperiodic time measurement are solely extraretinal, in others purely retinal, and in others both retinal and extraretinal receptors contribute. The 2nd major problem is that no extraretinal receptor involved in these responses has ever been identified. The evidence from localization experiments overwhelmingly points to the central nervous system, and in particular to the brain, as the locus of the photoreceptors; and it seems reasonable to assume then that photosensitive neurons are involved. But many of the detailed questions about the physiological properties of the photoreceptors as well as the mechanisms utilized in the transduction, processing, and transmission of the temporal information provided by the daily light cycle await a more precise identification of the cells responsible.

These problems not withstanding, there has been some speculation on the potential significance of extraretinal photoreception in entrainment and photoperiodic time measurement. It has been suggested, for example, that in holometabolous insects extraretinal pathways may be necessary to provide photic information during metamorphosis, a time when organized photoreceptive structures may be absent or not yet functional[51,52]. In other cases where
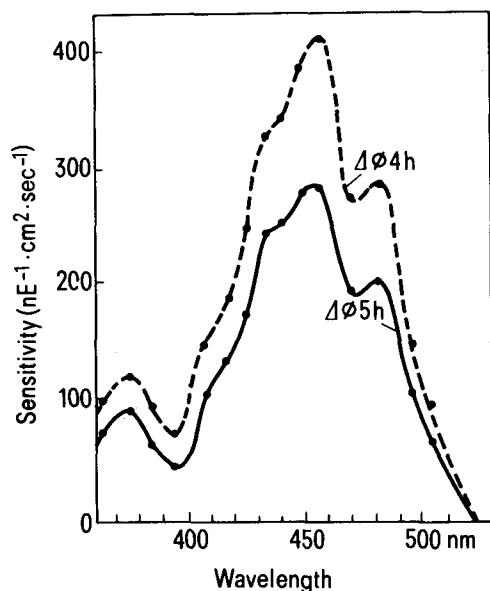

Figure 4. Action spectra for 4- and 5-h phase delays ($\Delta\emptyset 4$ h and $\Delta\emptyset 5$ h, respectively) in the eclosion rhythm of *Drosophila pseudoobscura.* The phase delays were evoked by a 15-min light pulse of a given wavelength presented at a standard phase of the circadian cycle[46].

both retinal and extraretinal elements contribute the photoreceptors may perform non-redundant functions. In molluscs, for example, extraretinal receptors may serve to expand spectral sensitivity or lower the threshold of response. Finally, it has been suggested that the widespread distribution of extraretinal photoreceptors might be a reflection of an early participation of a photopigment in the molecular machinery of the clocks that function in the daily and seasonal measurement of time (although a pigment is clearly not prerequisite for a clock)[52].

In summary, substantial progress has been made in pointing out a pervasive functional role for extraretinal photoreception in the temporal organization of a phylogenetically diverse group of organisms. The receptors are typically (though not invariably) sensitive to blue light and insensitive to red, located in the central nervous system, and are sensitive to low levels of illumination. Further studies promise to provide insight into remaining questions about the identity, properties and functional advantages of extraretinal pathways for the perception of the temporal information provided by the daily change in light intensity.

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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