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. 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 approximately, but rarely exactly, 24 hours. 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 ‘multioscillators’ in nature; that is, individuals possess more than 1 circadian clock. 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. 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 photosensitive on both neurophysiological and ultrastructural evidence. Although details of ultrastructure and innervation are beyond the scope of the present discussion a number of reviews can be consulted for additional information. 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 (pineal-fugal) innervation in lower vertebrates to a primarily efferent (pineal-loop) innervation in birds and mammals. All pineal organs show evidence of secretory capabilities. 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. Historically it was believed that the terminal enzyme in melatonin synthesis, hydroxindole-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. The participation of extraretinal photoreceptors (ERRs) in the entrainment of circadian rhythms in fish has been assessed in the eel Anguilla anguilla, the trout Salvelinus fontinalis, the lake chub Coeusius plumbeus, and the pencil fish Nannostomus beckfordi anomalous. In Anguilla neither blinding nor blinding combined with pinealectomy abolished entrainment of the circadian activity rhythms to LD 12:12 light cycles. 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. 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. The shift in sensitivity toward 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. 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.

The activity rhythms of fish under natural conditions at the arctic circle have significant implications concerning the nature of circadian organization. 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, Loa loa, and the lake chub, C. plumbeus, has significant effects on the period of the activity rhythm expressed in constant darkness. 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. 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. However, blinded newts Notophthalmus viridescens, which naturally lack a frontal organ, can still entrain to LD cycles. 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. 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 griseus, can orient in a predicted direction to the sun even if the eyes are removed. However, orientational ability is lost in blinded, but not sighted.