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 of 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 multioscillators 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 ubiquitously 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 photosen-
sory on both neurophysiological and ultrastructural
evidence1-13. Although details of ultrastructure and
innervation are beyond the scope of the present
discussion a number of reviews can be consulted for
additional information11-13. In general, however, the
photoreceptive cells in the pineals of lower vertebrates
decompose or lost in birds and mammals
concomitant with a shift in innervation from a pri-
arily afferent (pinealo-fugal) innervation in lower
vertebrates to a primarily efferent (pinealo-pedal)
inervation in birds and mammals. All pineal organs
show evidence of secretory capabilities14,15. Much
interest has focused on the ability of pineals to
synthesize a variety of indoleamines including 5-
methoxy N-acetyltryptamine (melatonin). A remark-
able feature of vertebrate pineals is the presence of
daily rhythms in enzyme activities and biochemical
concentrations, including melatonin15. Historically
it was believed that the terminal enzyme in melato-
nin 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 capabili-
ties including the retina and the Harderian gland16-22.

Fish
The participation of extraretinal photoreceptors
(ERRs) in the entrainment of circadian rhythms in
fish has been assessed in the eel Anguilla anguilla23,
the trout Salvelinus fontinalis24-25, the lake chub Coe-
sius plumbeus26, and the pencil fish Nannostomus
beckfordi anomalus27. In Anguilla neither blinding nor
blinding combined with pinealectomy abolished en-
trainment of the circadian activity rhythms to
LD12:12 light cycles23. 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 year24,25. 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 sen-
sitivity at 538–568 nm) than in blinded or blinded-
pinealectomized fish in which entrainment was
restricted to 568–742 nm26. 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 brain28. Also the intensities of light
required to entrain blinded or blinded-pinealecto-
mized 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 blinding27.

The activity rhythms of fish under natural conditions
at the arctic circle have significant implications
concerning the nature of circadian organization29-31.
Activity patterns switch from diurnal to crepuscular
during which 2 activity components are clearly pre-
sent 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 dark-
ness32,33. Typically pinealectomy of freerunning fish
can cause changes in the period of the rhythm as well
as an increased variabilty 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 ac-

tivity rhythms in amphibians34. Both intact and blind-
ed 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
entrainment35. However, blinded newts Notophthal-
mus viridescens, which naturally lack a frontal organ,
can still entrain to LD cycles36. 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 recep-
tors can mediate compass orientation in amphi-
bians34. Many amphibians can steer in a partic-
ular 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 removed37. However,
orientational ability is lost in blinded, but not sighted