

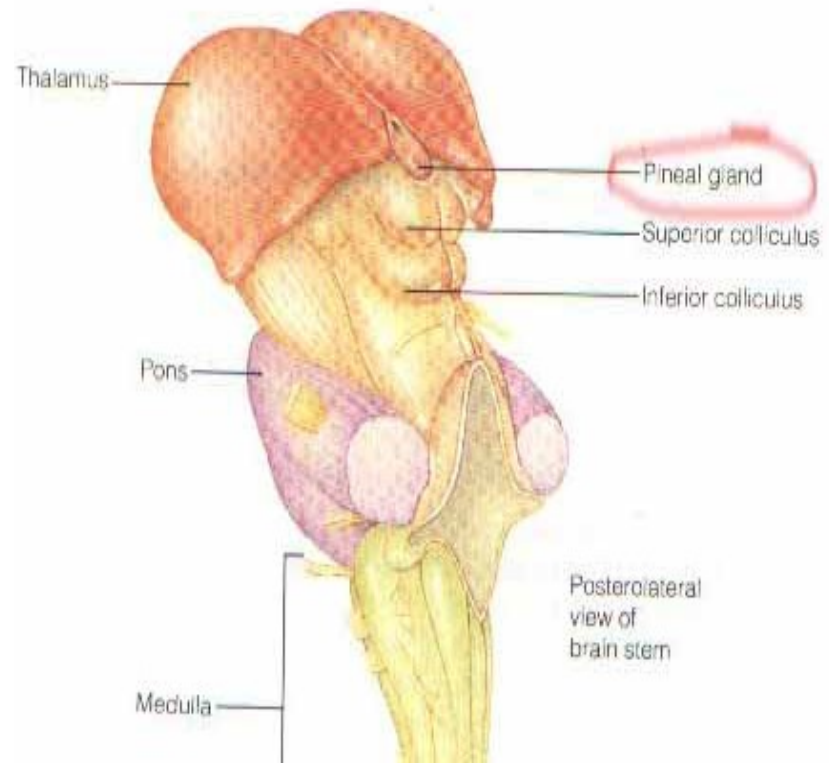
Pineal : as a clock???

# Pineal

- Endocrine Status by Otto Heubner a German pediatrician
- Epiphysis Cerebri
- Third Eye – Light transducing ability
- Photoneuroendocrine Transducer- Modulates physiological systems according to circadian rhythms
- Made of same tissue as the eye
- Well differentiated photoreceptive organ in lower vertebrates – a functional third eye
- Semi receded in higher vertebrates - migrated from position above to position below and center

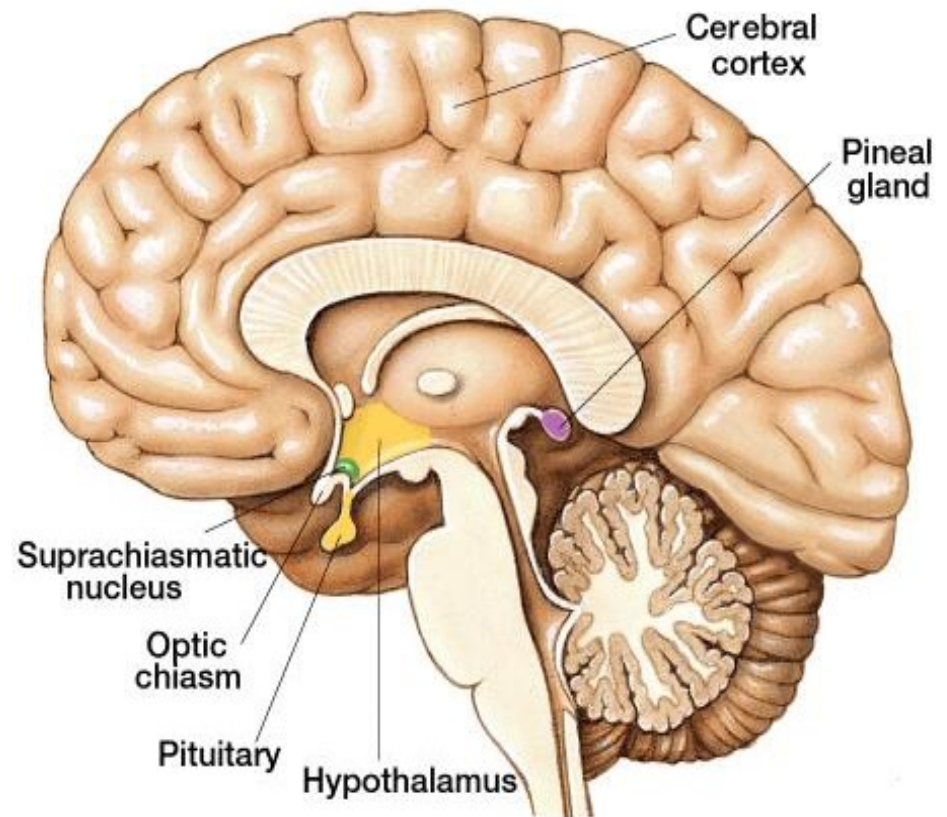
# Pineal

- Small, reddish gray, vascular, conical body of rudimentary glandular structure
- Secretes melatonin and serotonin depending on photic cues.

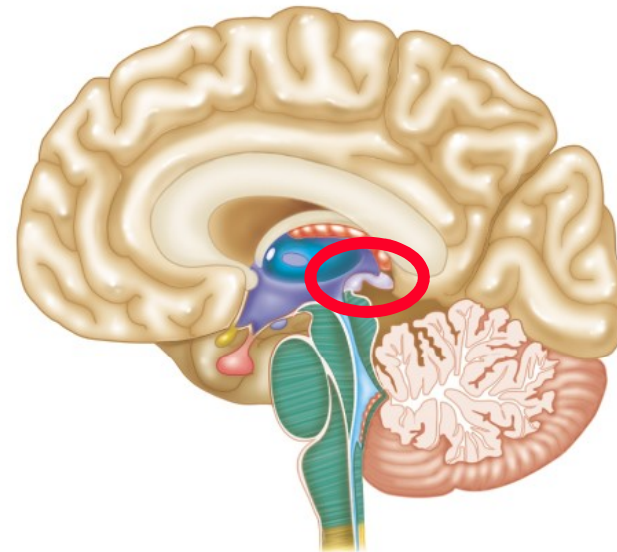
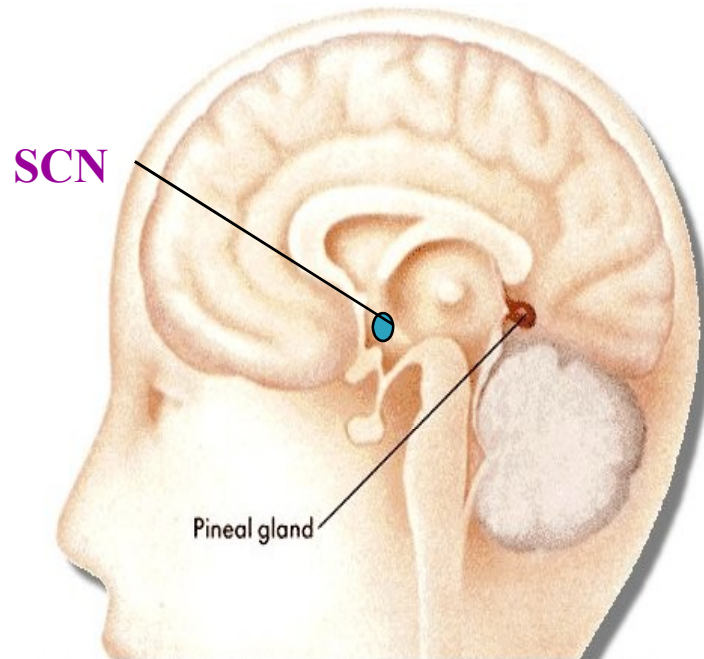


# Anatomy

- Center of the brain behind and above the pituitary gland
- Directly behind the eyes.
- Attached to the roof of 3<sup>rd</sup> ventricle
- No direct connection to brain



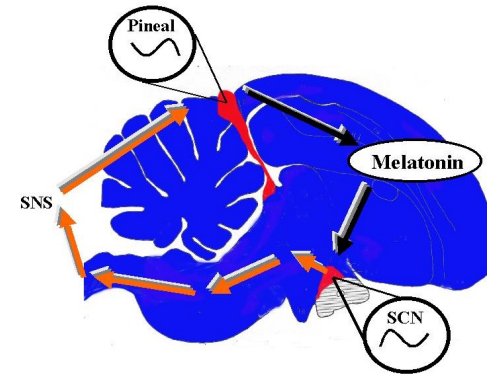
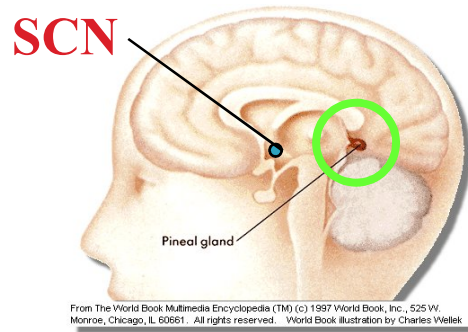
# Pineal gland and SCN in the human brain



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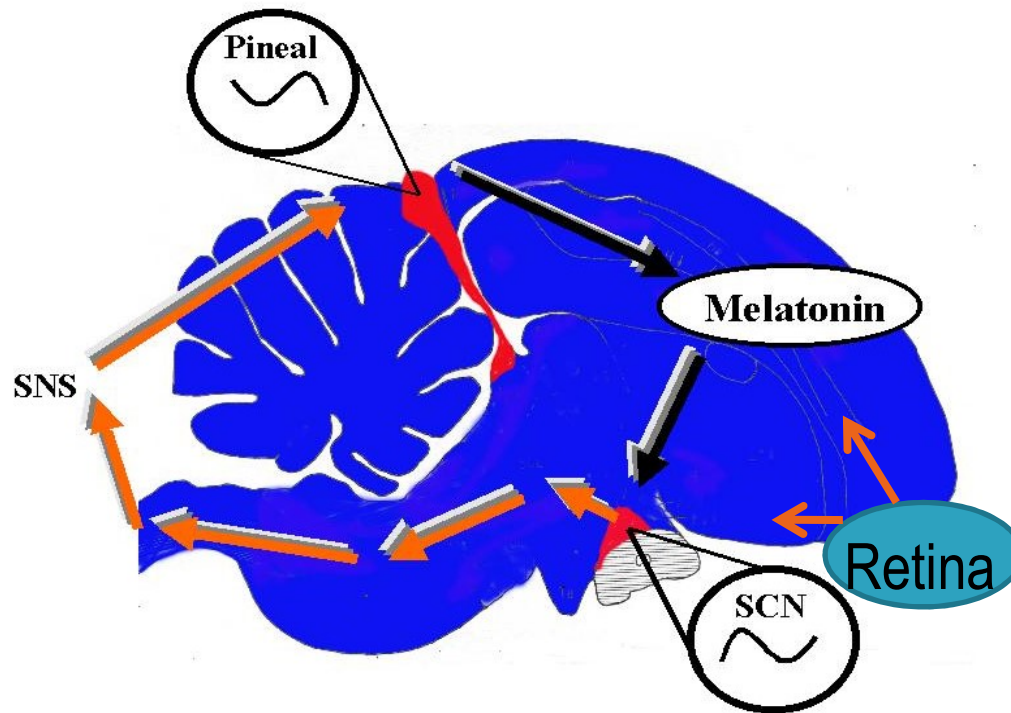
# The PINEAL



**Pineal itself is a clock, at least in the non-mammalian vertebrate**

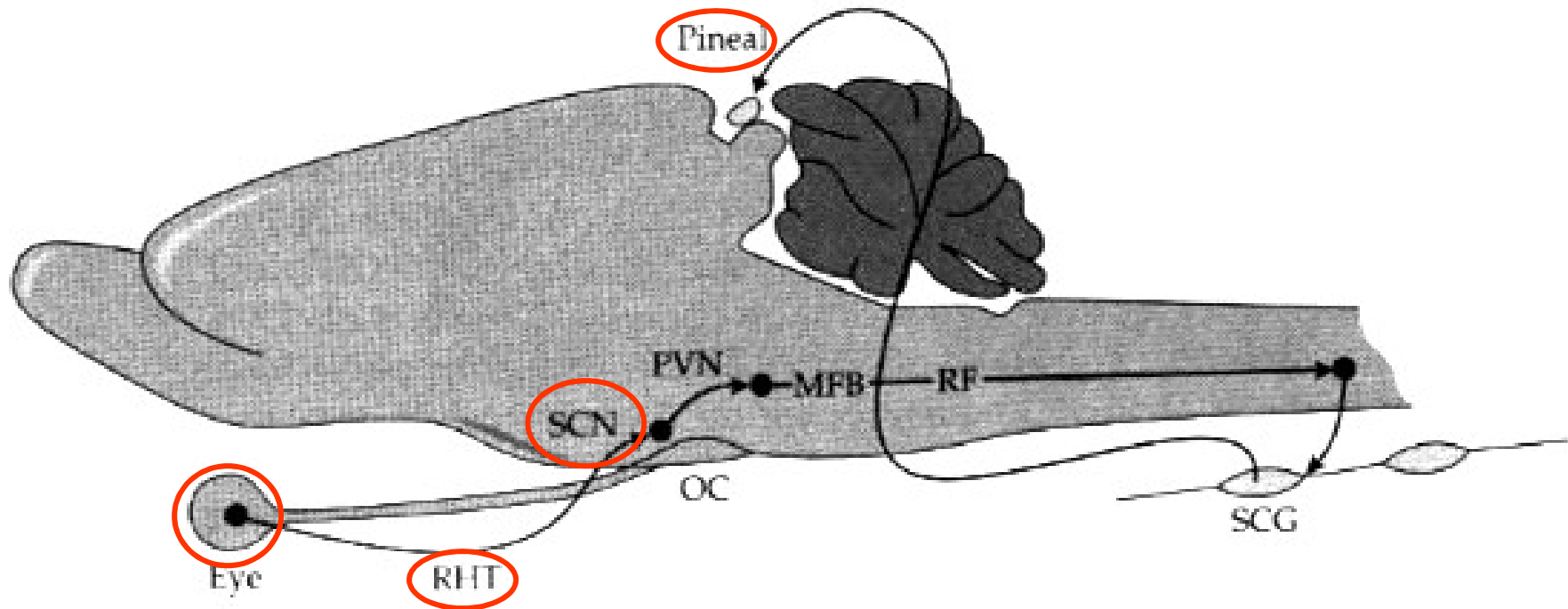
***Important in regulation of circadian and seasonal behaviour via melatonin secretion***

## The timekeeping system of birds



Relative contribution to overall pacemaker rhythmicity differ widely among species.

# The timekeeping system of mammals



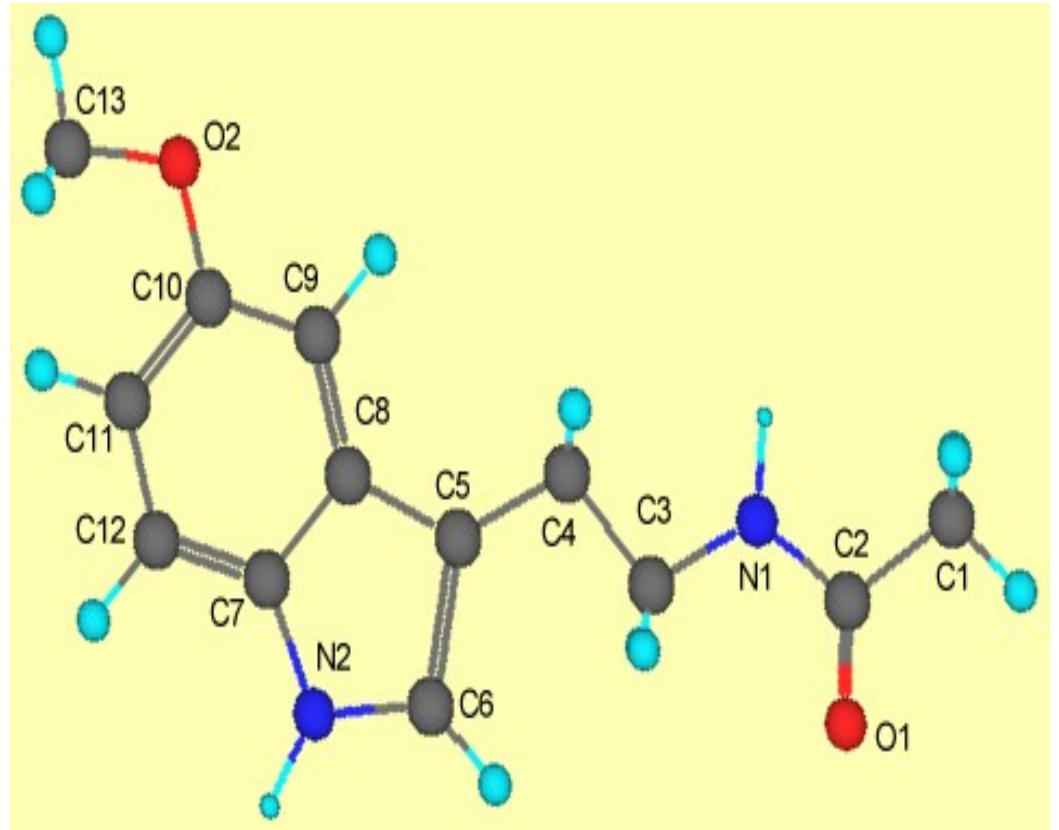
- Individual cells in SCN generate rhythms
- Eyes perceive LD-cycles with specialized 'circadian' photoreceptors
- Retino-hypothalamic tract (RHT) transmits photic information to SCN
- Suprachiasmatic nuclei (SCN) in hypothalamus are active during day
- Pineal gland is active at night and produces melatonin which inhibits SCN



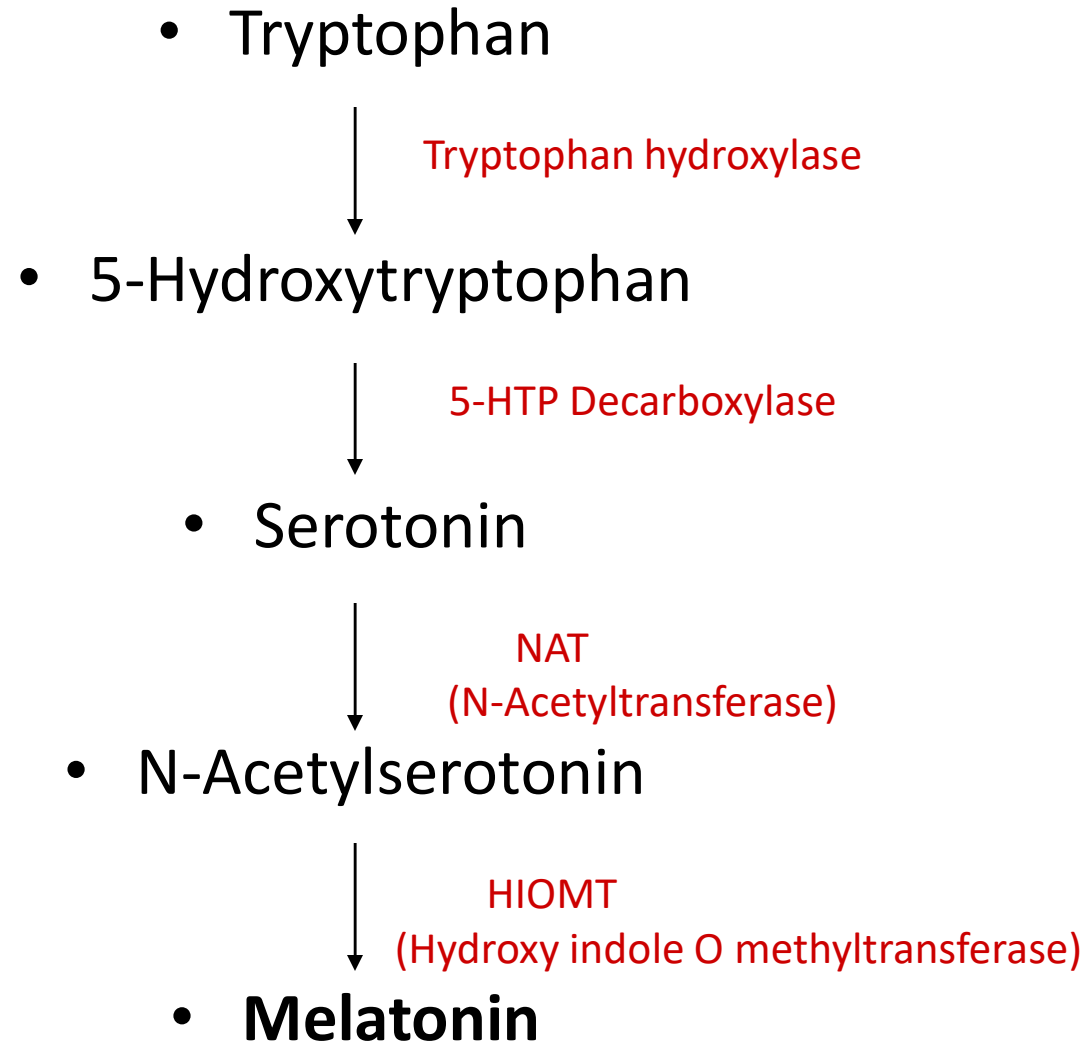
# Melatonin

# What is Melatonin?

- **N-acetyl-5-methoxytryptamine (Indoleamine)**
- **Empirical formula:**  
 $C_{13}H_{16}N_2O_2$
- **Color: pale yellow**
- **Normal state: crystalline solid**
- **Precursor: serotonin**
- **Synthesis and secretion dependent on light exposure to eyes**



# Biosynthesis of melatonin



# Metabolism

- Metabolized by the liver
- Converted to sulfate or glucuronide for urinary secretion

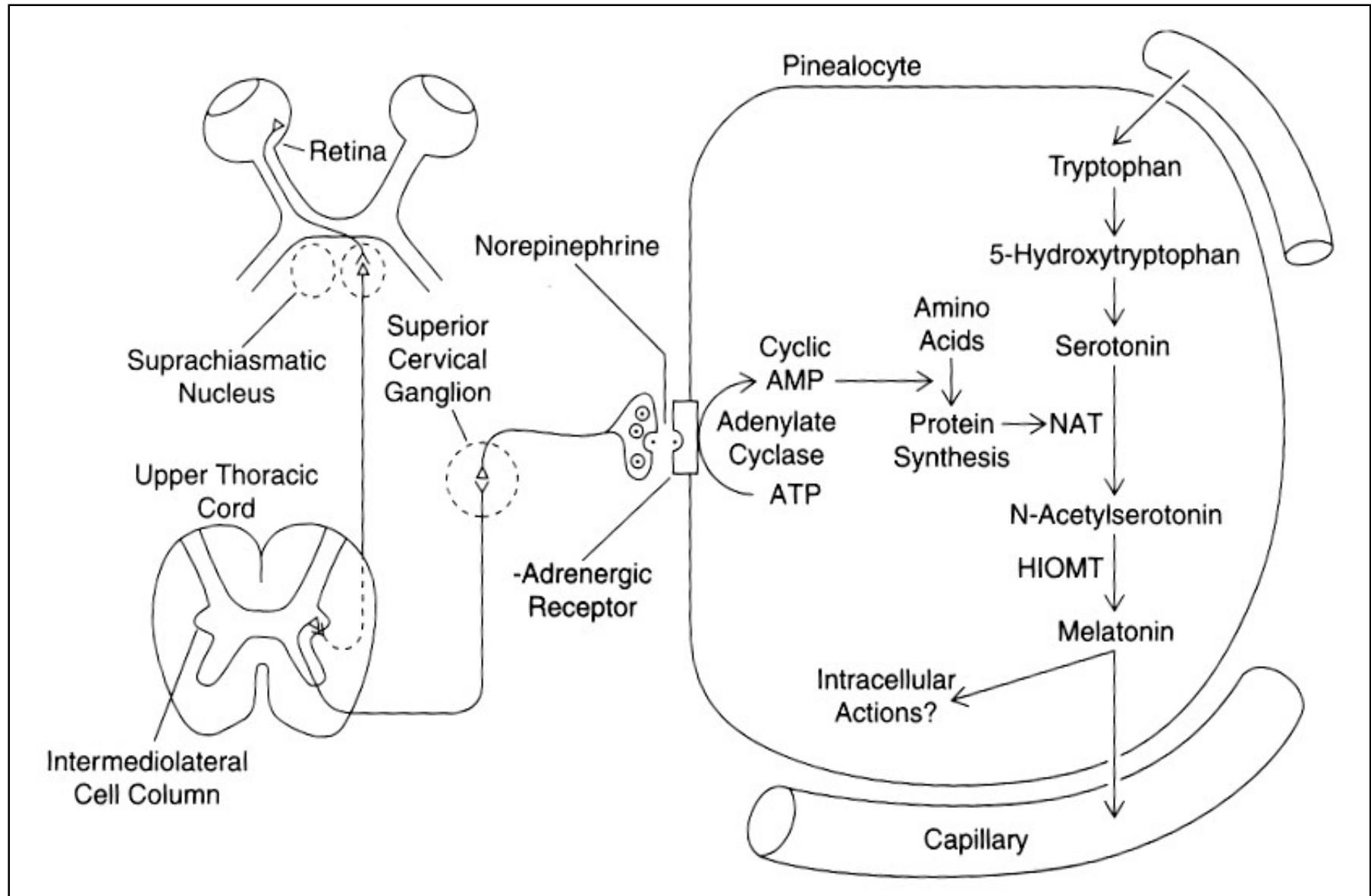
Melatonin is a lipophilic compound; freely diffuses through biological membranes, and readily crosses hemato-encephalic barrier, hence ubiquitous in nature.

Half life of melatonin is ~10 min.

Metabolized primarily in the liver by hydroxylation to 6-hydroxymelatonin, which is converted to sulfate or glucuronide.

Also deacetylated to 5-methoxytryptamine, which is deaminated into 5-methoxyindoleacetic acid and 5-methoxytryptophol.

# Melatonin secretion and circulation



Photosensitive cells in the retina detect light and directly signal the suprachiasmatic nuclei (SCN), entraining its rhythm to the 24-hour cycle in nature.

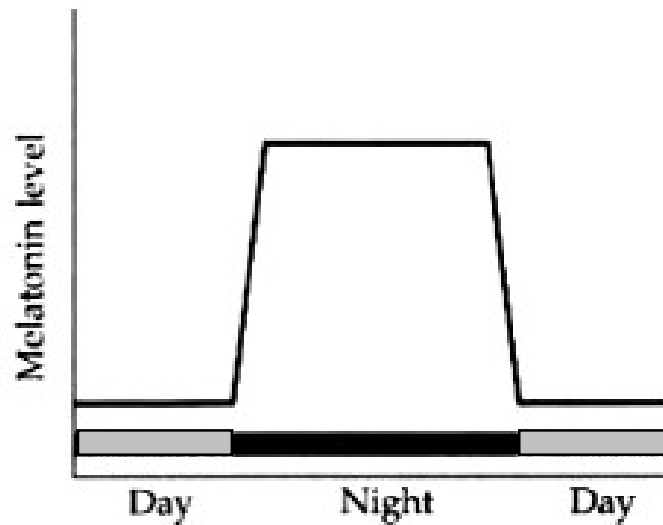
Fibers project from the SCN to the paraventricular nuclei (PVN), which relay the circadian signals to the spinal cord and out via the sympathetic system to superior cervical ganglia (SCG), and from there into the pineal gland.

# Melatonin biosynthesis

- Pineal gland is under noradrenergic control
- During the day, light blocks norepinephrine release, inhibiting melatonin synthesis and increasing the level of serotonin
- At night, nerve fibers connected to the pineal gland release norepinephrine and stimulate the synthesis of melatonin
- Effects through  $\alpha$  and  $\beta$  adrenergic receptors

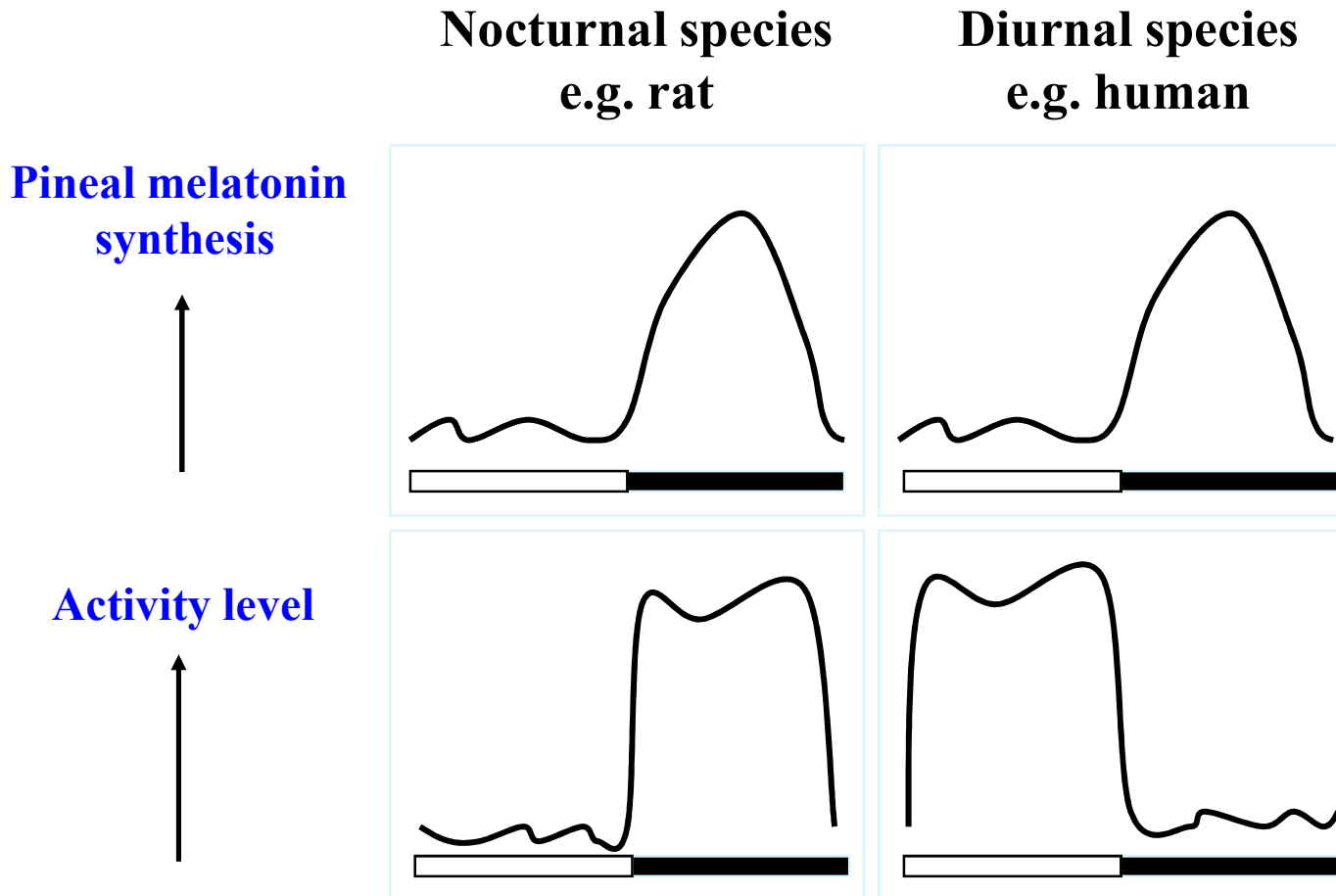


Melatonin is only produced at night ! “The chemical messenger of dark”



Regardless the animal is diurnal, nocturnal or crepuscular

# Melatonin and activity - phase relationship in nocturnal and diurnal species

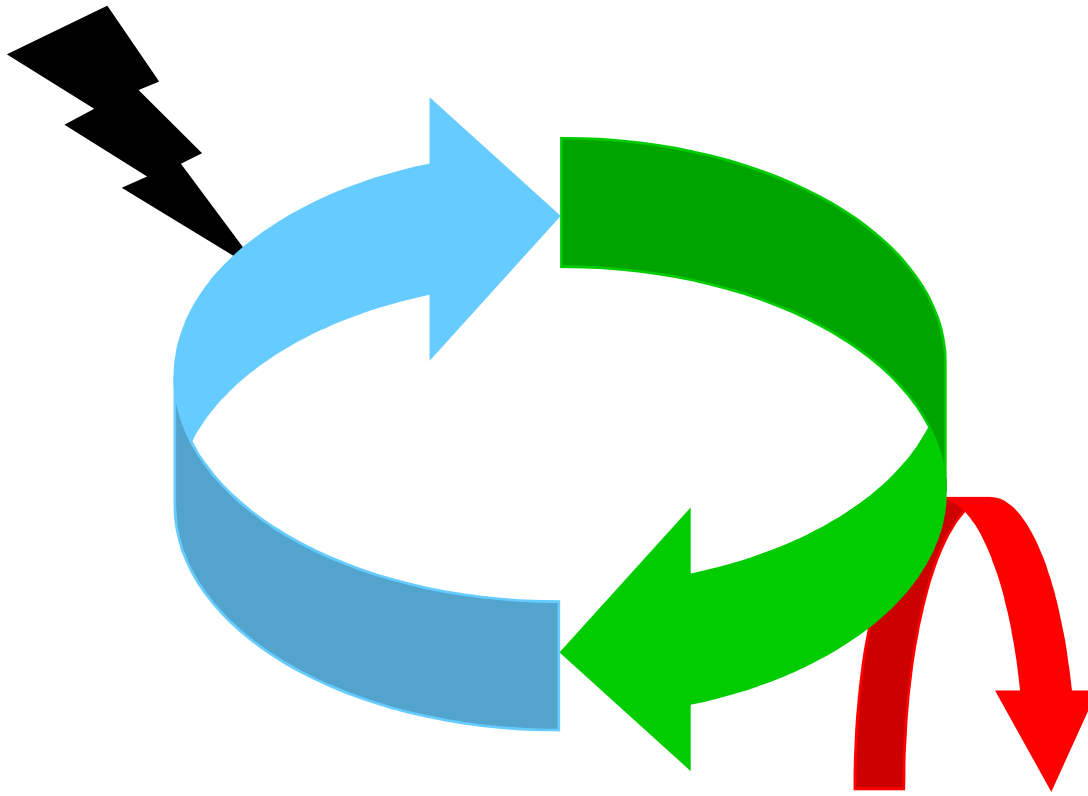


The presence of melatonin is demonstrated in almost all groups of organisms, from plants, protozoa to people.

So, the pineal gland is not the prerequisite structure to make the melatonin.

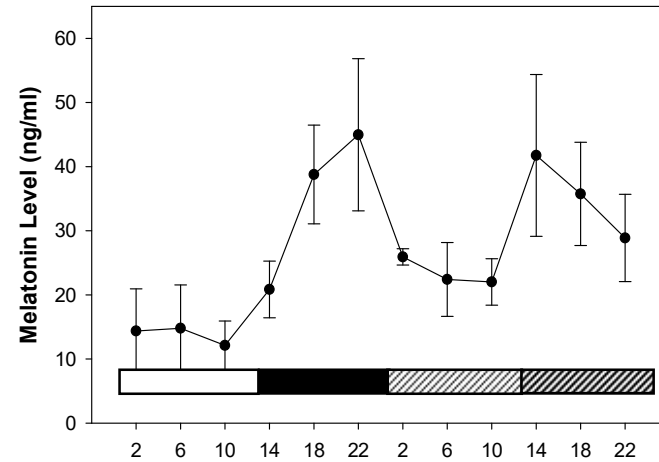
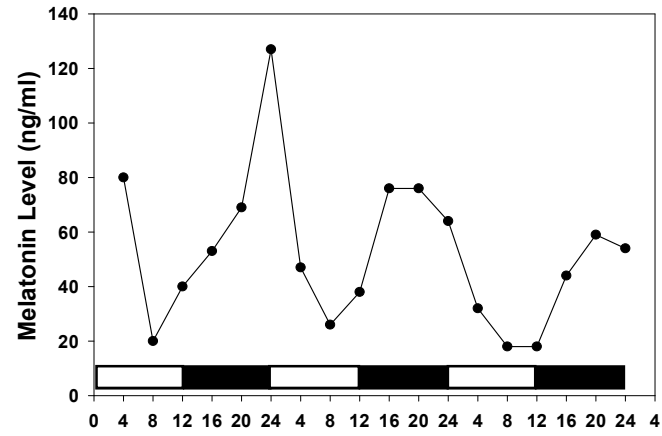
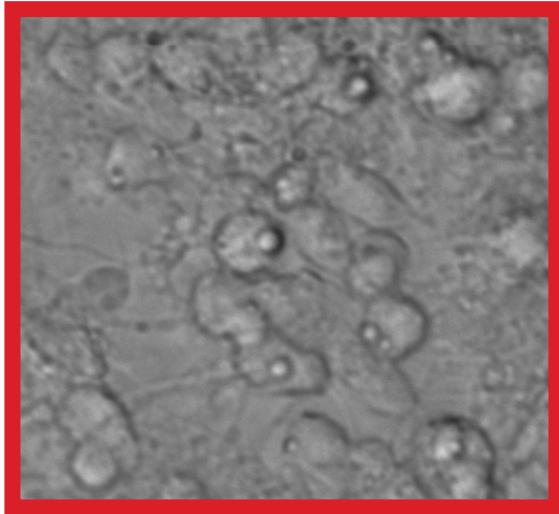
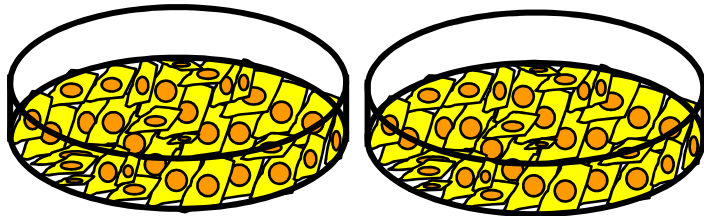
The molecule of melatonin seems to have been evolutionarily conserved.

**Is pineal a clock?**



**YES**

# Pineal cells contain self-sustained oscillators



# Melatonin receptors

- Two receptors in mammals – Mel1A and Mel1B
- G-protein coupled cell surface receptors
- A third receptor found in frogs and birds but no mammalian homolog has been found
- Highest density of receptors found in SCN of the hypothalamus, anterior pituitary and retina

## Why pineal is considered to be a neuroendocrine transducer?

Melatonin is synthesized in the Pineal by the conversion of serotonin, in response to norepinephrine released from postganglionic neurons from the superior cervical ganglia, thus the Pineal gland is considered to be a neuroendocrine transducer, as neural input to Pineal is converted into an endocrine output.

# Pineal and Environment

a) Light: dark cycle

**Does light: dark cycle affect the characteristics of the melatonin rhythm?**

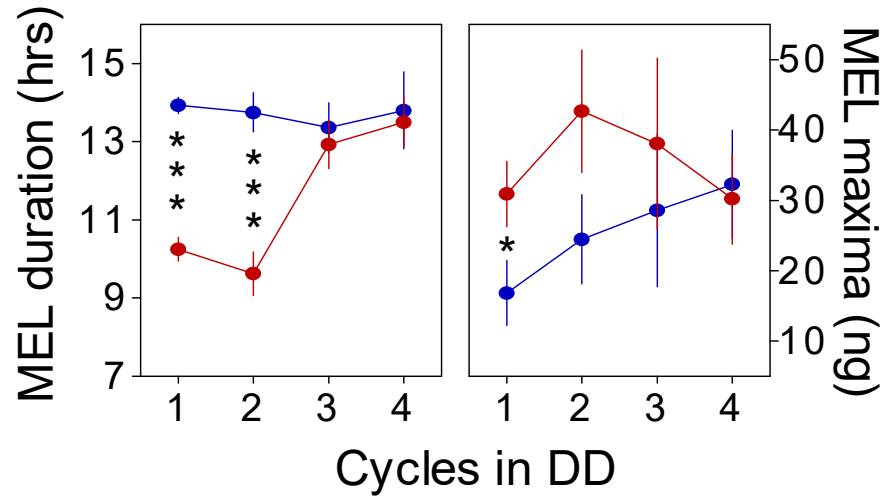
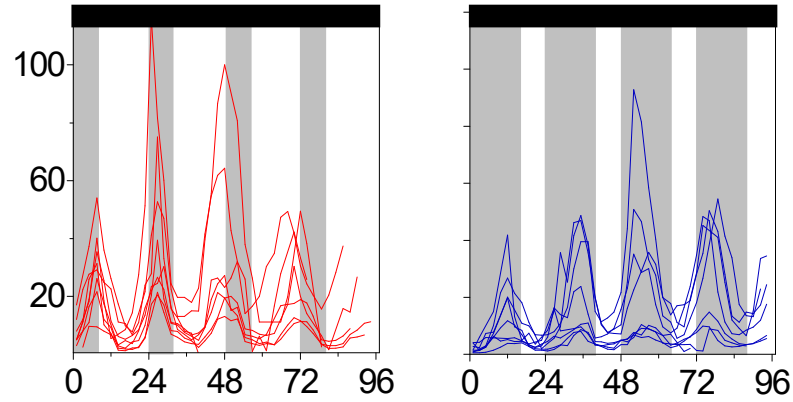


# Evidence 1

## *in vitro*: Pineal gland

LD 16/8 hrs

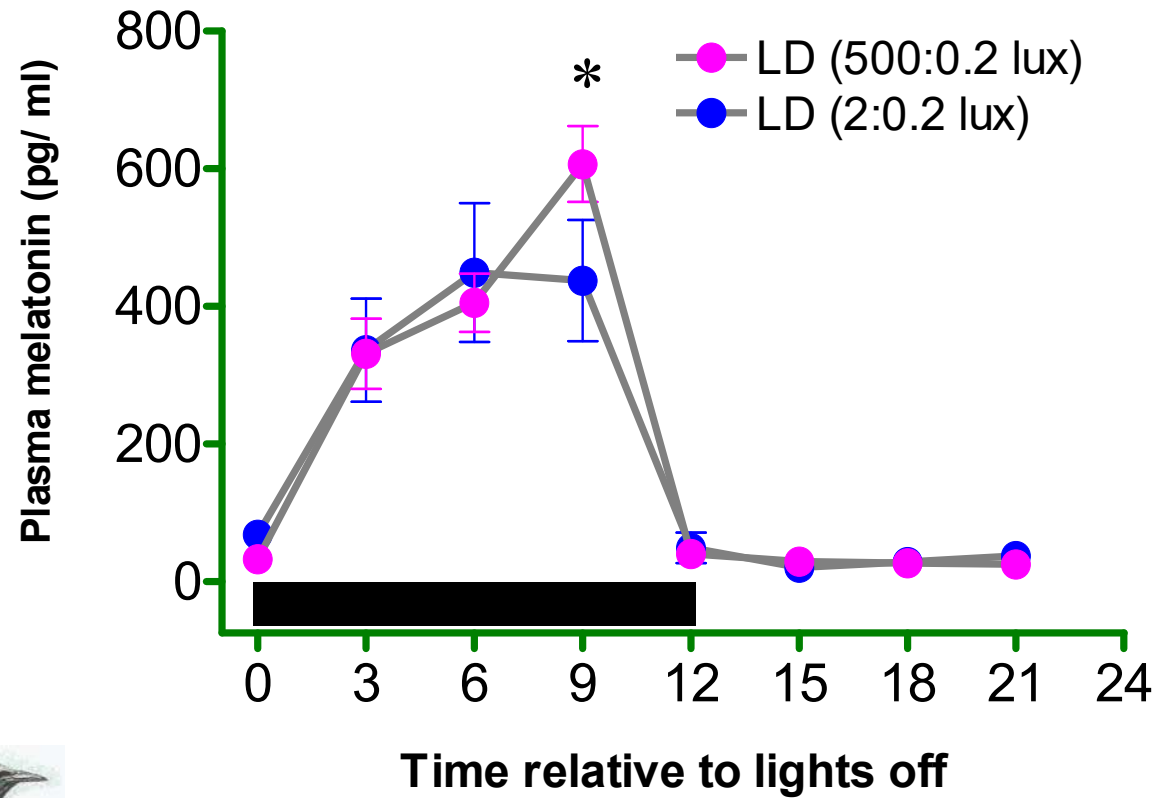
LD 8/16 hrs



*PNAS 2000*

## Evidence 2

### European starling (*Sturnus vulgaris*)

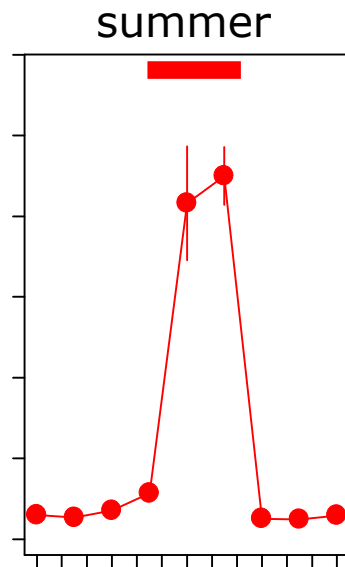
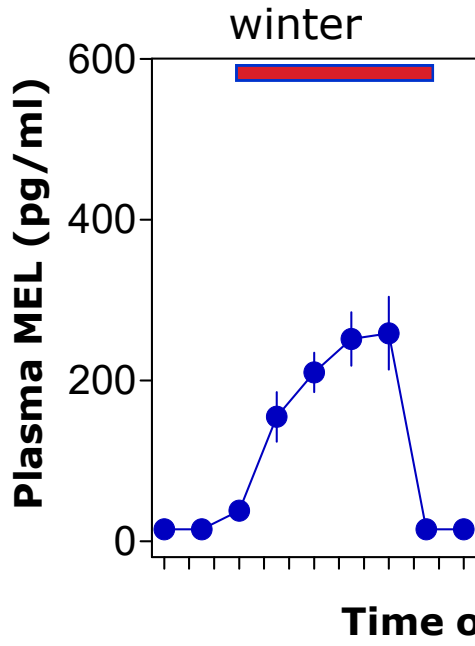


*J. Comp. Physiol.* 2000

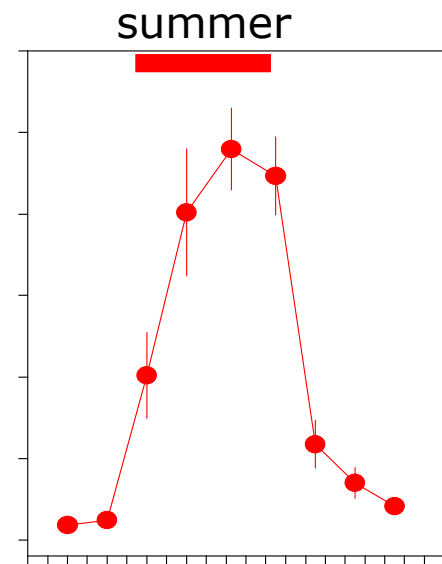
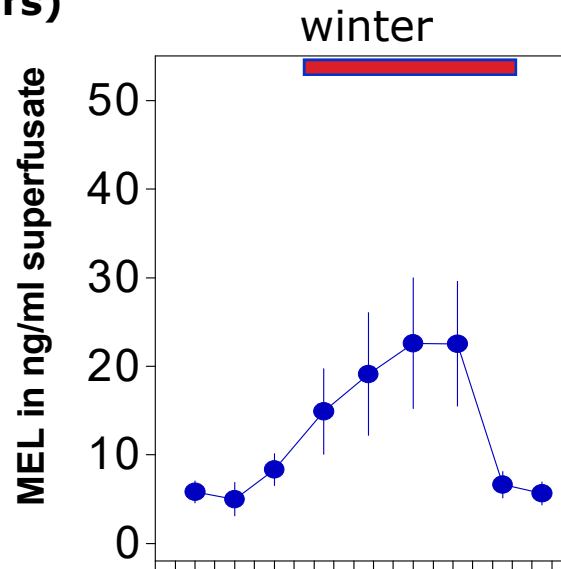
# Pineal and Environment

## b) Season

**Does season affect the characteristics of the melatonin rhythm?**



Time of day (hrs)



Time in culture (hrs)

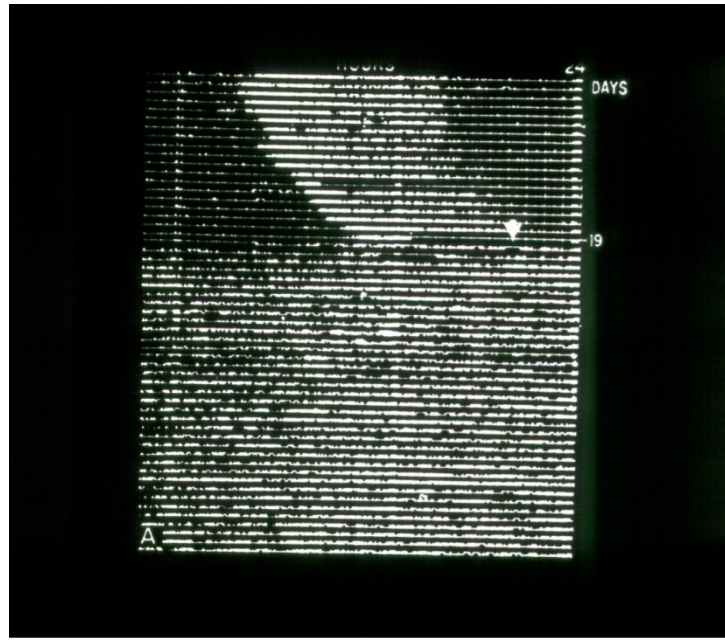
# Physiological roles of melatonin

## **Two major physiological roles of melatonin**

- 1. is involved in circadian organization.**
- 2. is critical for time measurement.**

**Pineal is involved in circadian organization**

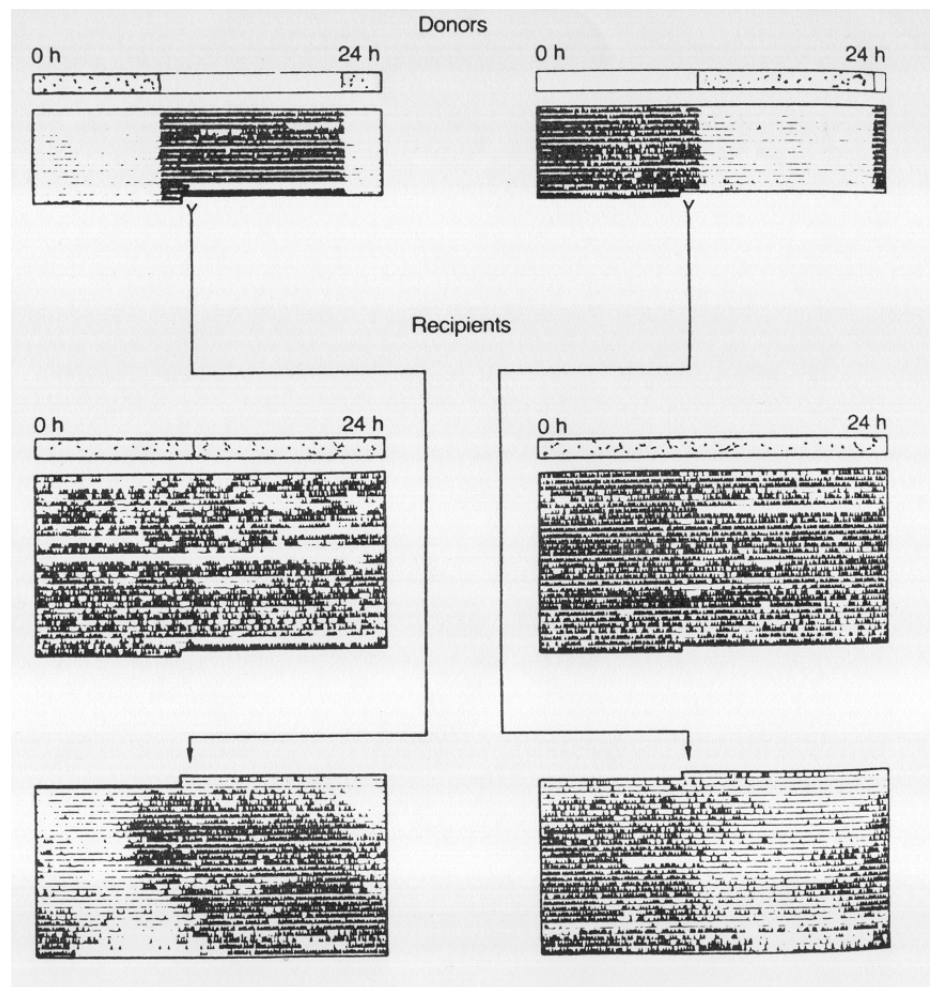
**Effect of pineal removal!!**



Surgical removal of  
the pineal gland in house  
sparrows abolishes  
circadian rhythms

Gaston and Menaker, 1968



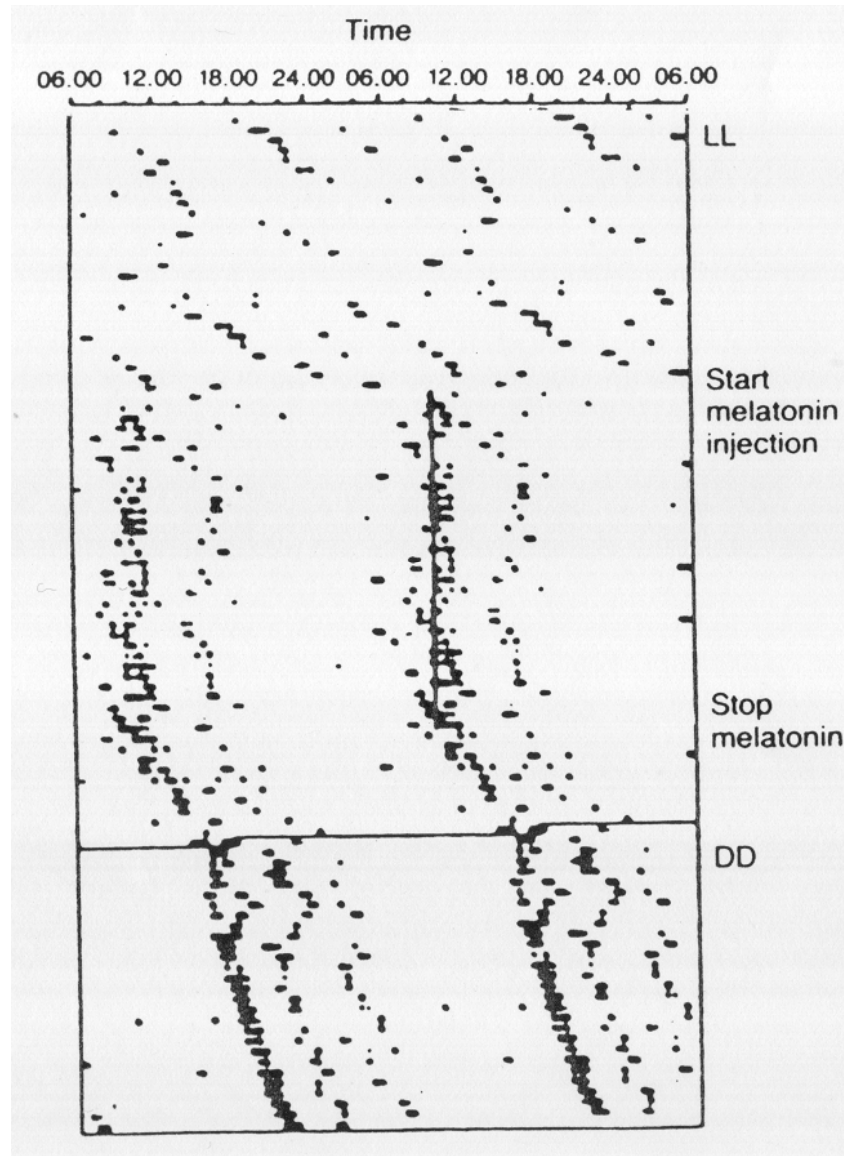


Transplantation of pineal transfers the phase of the donor to pinealectomized English sparrows, *Passer domesticus*, whose circadian rhythms of activity are abolished by pinealectomy (Zimmerman and Menaker 1979)

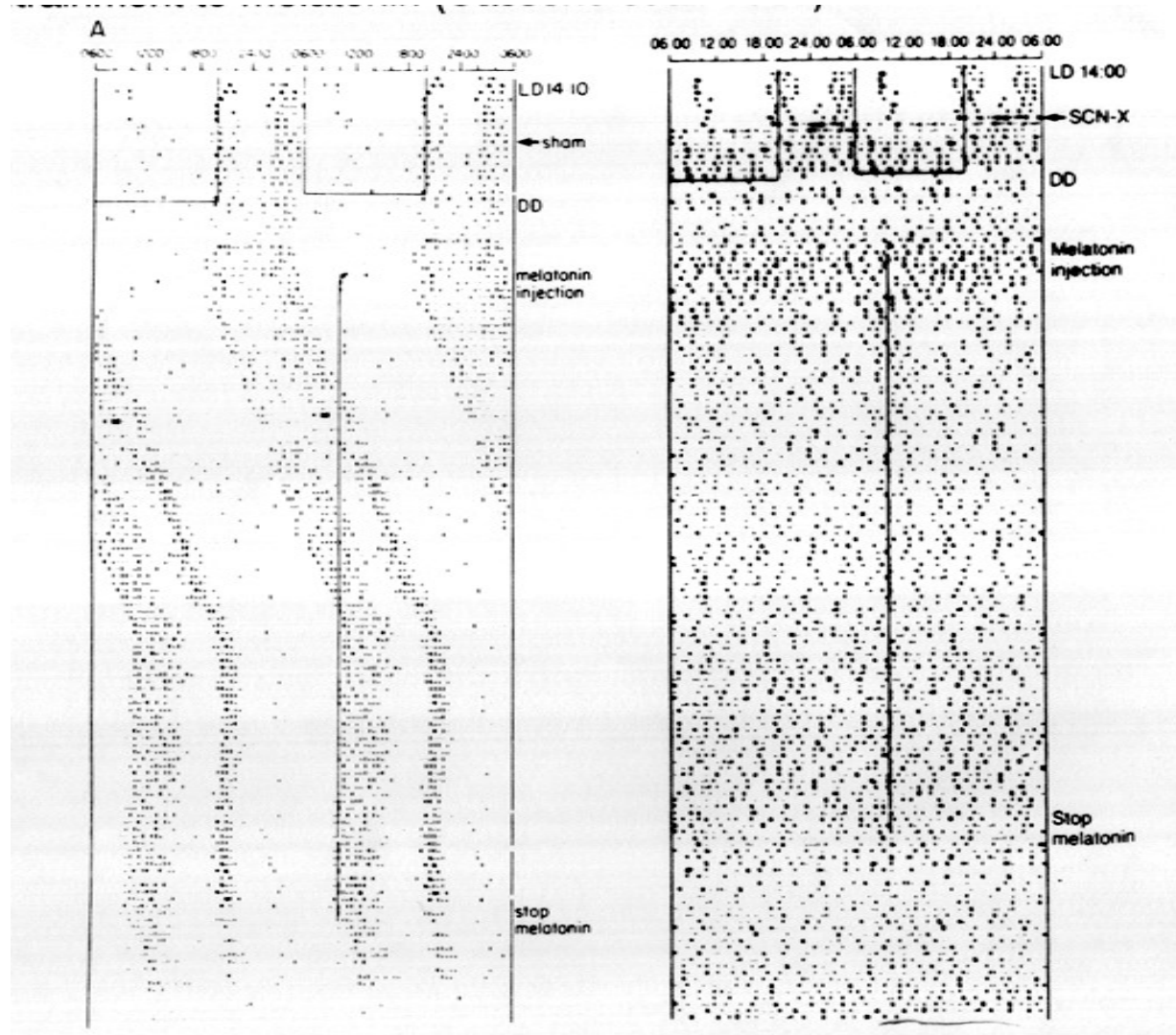
**Pineal is involved in circadian organization**

**Effect of melatonin administration**

# Entrainment of disrupted activity rhythm in rats in LL



The ability of melatonin to entrain the circadian activity rhythm requires an intact circadian pacemaker (the SCN); SCN lesioned animals fail to show entrainment to melatonin (Cassone et al. 1986)

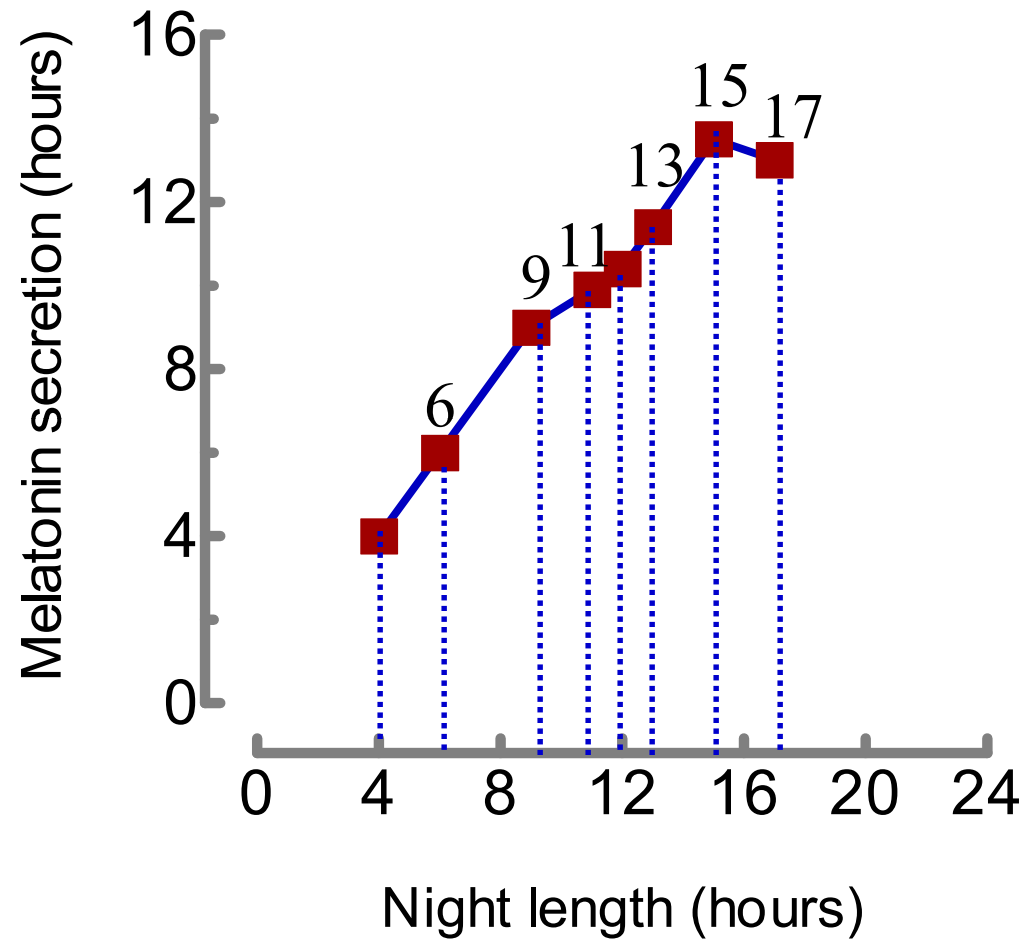


# **Pineal and photoperiodism**

## Pineal gland: Possible involvement in time measurement

- Pineal melatonin secretion is regulated by light through a multi-step nervous pathway.
- Exclusively nocturnal
- Repeatable rhythmicity
- Conveys the photoperiodic information through the duration of its secretion

# Melatonin release is photoperiod dependent

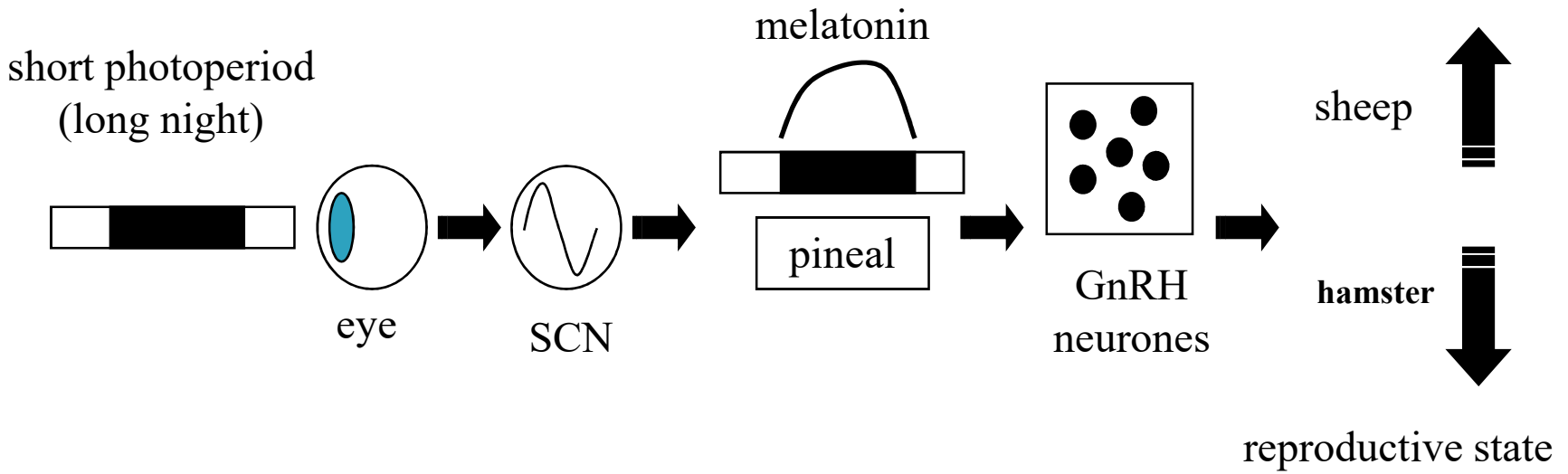
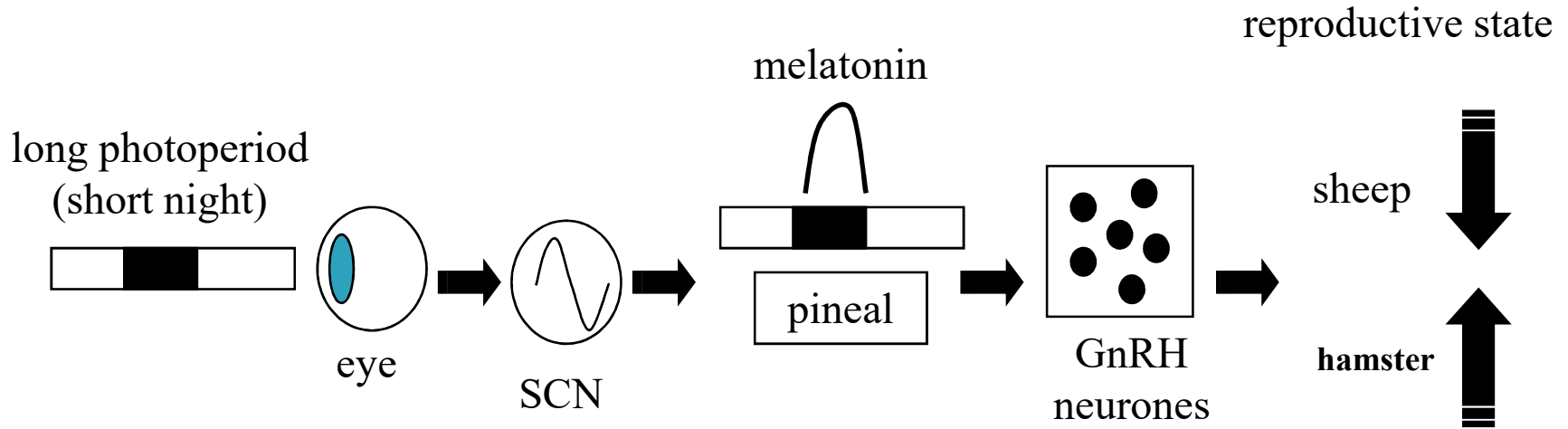


## Pineal gland: Response to changes in day-length

Complex set of mechanism involves:

- Detection of presence of melatonin above a minimum threshold (night vs day)
- Detection of duration of presence of melatonin above this threshold (long day vs short day)
- Detection of changes in the duration of melatonin presence related to previous melatonin exposure (increasing vs decreasing daylength)

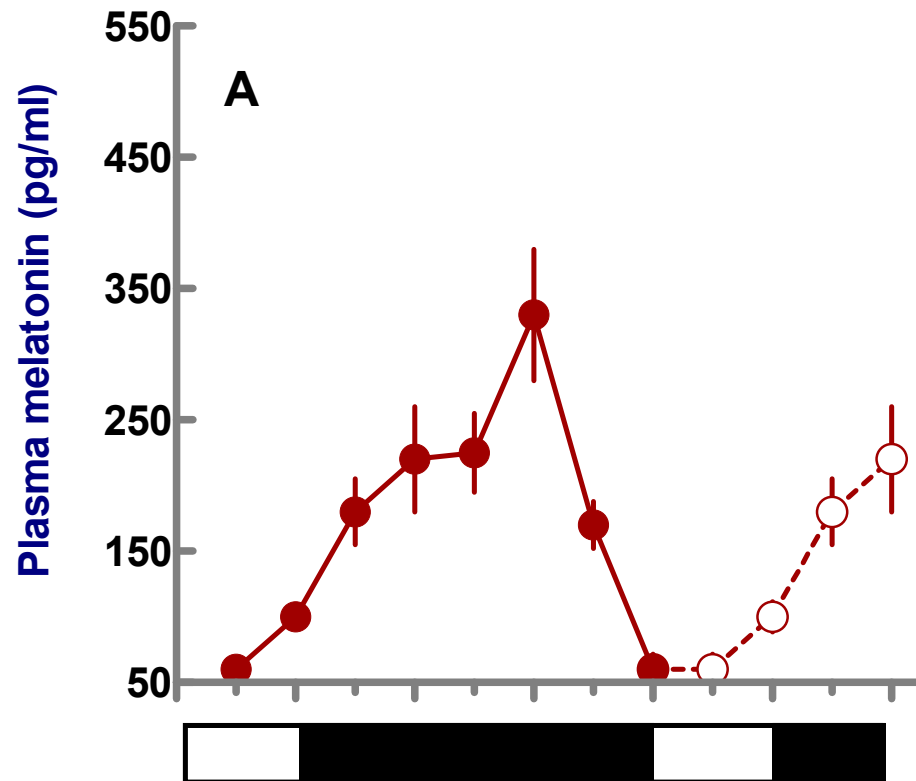




# Pineal Role in Reproduction

- Pineal Melatonin Mediates Photoperiodic Time-Measurement in Mammals
  - Pinealectomy Abolishes Seasonal Cycles (long-day and short-day breeders)
  - Timed Melatonin Infusions Restore Them
- However, While the Pineal Gland is Important for Circadian Rhythms in Birds, and Circadian Rhythms are Important for Photoperiodism, **relatively Little Effect of Pinealectomy Has Been Found For Gonadal Cycles in Birds**

# Melatonin: the sleep master



# Seasonal changes in the reproductive tract and behavior

- Shrinkage of testes in hamsters during non-breeding winter seasons correlate with levels of melatonin
- Laboratory manipulations can change reproductive activity and
- Administering melatonin to a neonate can cause developmental change: pre-programming effect of melatonin

# Biological effects

- Melatonin the sleep master
- Regulates neuroendocrine system
- Regulates circadian sleep-wake cycle
- Controls functions such as metabolism, reproduction, appetite, balance, immune system and excessive free radical activity

# Antigonadal properties

- Overproduction of melatonin leads to delayed puberty and underproduction leads to precocious puberty
- Effect through melatonin receptors on gonadotrophs of anterior pituitary
- Androgens and estrogens may directly regulate the production of melatonin in the pineal gland
- Melatonin inhibits secretion of gonadotropic hormones, leutinizing hormone and follicle stimulating hormone from the anterior pituitary

# Biological Clocks

- Melatonin levels correspond with light-dark cycles
- In mammals melatonin has a modulatory role and works as synchronizer to prevent loss of synchrony not a driver of rhythm
- Pinealectomy of rodents leads to disruption of circadian system
- In humans pharmacological suppression of melatonin enhances magnitude of light induced phase shifts

# Chronobiology

- Melatonin is implicated in circadian thermoregulation
- Melatonin and core body temperature rhythms are closely coupled, the nadir of temperature occurs within 1h of melatonin peak



# Effects of melatonin on skin pigmentation

- Normal background response is controlled by presence or absence of MSH and not under the control of the pineal gland
- Melatonin lightens skin pigmentation, in amphibians melatonin from pineal causes melanosome aggregation within dermal melanophores causing color change 'blanching'
- Body blanching eliminated by pinealectomy and mimicked with supplementation

# Retinal melatonin

- In fish and amphibians, pineal is directly light sensitive due to its location on the surface of the brain
- In birds and reptiles, the signal is usually a combination of direct photoreception and light induced hormonal signals
- In humans, due to the location deep in the midbrain, the pineal gland function is extensively regulated by signals arising from the retina

# Medical potential

- Jet lag and shift work
- Sleep disorders: DSPS, insomnia
- Aging
- Antioxidant
- Cancer
- Immunity
- Endocrine disorders
- Schizophrenia

# Jet lag and shift work

- 5mg melatonin administered to shift workers at bedtime increased alertness during waking hours
- Phase advances observed when administered in the late afternoon
- Equally effective to bright light exposure

# Sleep disorders

- Melatonin is given by researchers during day rather at night
- Delayed Sleep Phase Syndrome (DSPS)  
Common in adolescents  
People sleep late at night or early in the morning

Melatonin may be a help for this condition

# Aging

- Circadian rhythm not present at birth in mammals
- 3-4 months of age discernible melatonin level
- After puberty significant drop in nocturnal melatonin levels
- Recent studies show melatonin can be used as an anti-aging hormone
- Dietary restriction preserves rhythmic melatonin level and increases life span in mice
- Positive correlation in some studies

# Antioxidant

- Melatonin acts as an antioxidant by scavenging the hydroxyl radicals, peroxynitrite anions, peroxy radicals, and the superoxide anion radical
- Increases mRNA levels of antioxidative enzymes
- Protects DNA and mitochondria from oxidative damage

# Cancer

- Oncostatic effects reported on some human cell lines
- Melatonin appears to have antitumor activity
- Pinealectomy increases incidence of tumors
- Estrogen-related cancers in female are correlated to age of puberty
- Hypothesis of lower risk of breast cancer in blind women



# Immunity

- Modulation of immune response via circadian release of neurohormone melatonin
- Neuropeptides, neurotransmitters and the melatonin have significant response on the immune system
- Modulate antibody production and response to mitogen
- Products of immune system have substantial influence on the pineal gland
- Thymus one of the main targets of melatonin and its immunoenhancing effects may be mediated by opioids from T-helper cells and lymphokines

# Endocrine disorders

- Melatonin may be used in the treatment of postmenopausal osteoporosis
- Melatonin regulates calcium and phosphorous metabolism
- Menopause associated with decline in melatonin and increased pineal calcification

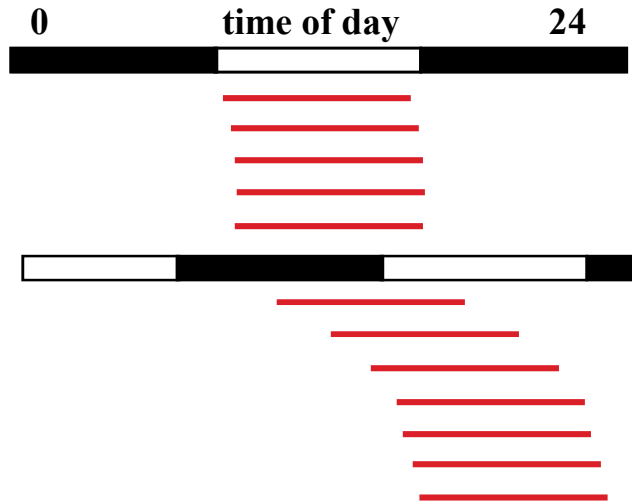
# **Is melatonin a clock- input or clock-output?**

**Melatonin is a reliable  
circadian output!!**

**? Whether the melatonin is  
an output or input, or both!!**

**Melatonin appears to be integral component of biological clock system in addition to its role in several non-clock functions.**

# Melatonin and jet-lag...



**Resynchronization takes time...**



Jet lag symptoms: sleep disruption, disruption of digestive processes, impaired psychological processes (e.g. attention, perception and motivation), general feeling of malaise



