PHYSIOLOGICAL EFFECT OF LIGHT.

The circadian system is much less sensitive to light than the visual system
(30 times in hamster, even 1000 in humans, long signals are necessary).

It responds to Δ light, not specific patterns.

In **mammals**, eyes are essential for the circadian rhythm. Not in the other vertebrates (evolutionary reason?).

Mammals: **light receptor neurons** different from cones and rods.

1% of ganglion cells (specific) → RHT (retino-hypothalamic pathway) → SCN

Opsin was initially found in certain ganglion cells of salmon's retina. Subsequently, **melanopsin** was discovered in mammalian ganglion cells (distributed throughout the cell membrane).

But **rods and cones also have a role**, probably important in low illumination and to sample different aspects of the visual scene.

Experiments in mice:

- Mice without cones/rods → entrainment by light

- Normal mice with decreased melanopsin → attenuated response

- Mice without cones/rods and with decreased melanopsin → no entrainment by light.

INTRINSICALLY PHOTOSENSITIVE RETINAL GANGLION CELLS (ipRGCs).

Summarized from Do and Yau, *Physiol Rev* 2010 and Do, *Neuron* 2019.

a) **Melanopsin** (Fig. 2A): G-protein coupled receptor;
plasma membrane;
very low density (< 1000 times lower than for opsins in rods and cones)
strong response to a single photon

b) **Single-cell response** (Fig. 2C): depolarized cells (close to threshold),
light-induced depolarization leads to A.P. firing.
Mechanism different from rods and cones, more similar to the invertebrates'

Melanopsin activates PLCβ4 by G-protein activation,
which leads to TrpC6-TrpC7 opening (non selective for cations)

Messenger: uncertain (likely a PIP_2 metabolite, but apparently not IP_3).

c) **Sustained response** (Fig. 2C, 8)
Integration of the light response during a long time.

d) **PACAP** (pituitary adenylyl cyclase activating protein).
Not found in classic retinal ganglion cells.
Its receptor is expressed in the target neurons of ipRGCs.

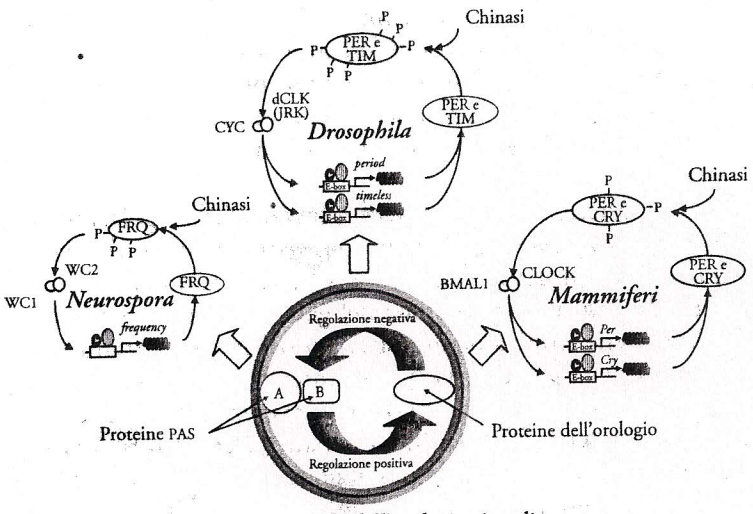
e) **Target structures of ipRGC fibers**.

Hypothalamus: SCN (photoperiodic entrainment) and pre-optic area (VLPO, sleep).
Thalamus: IGL (intergeniculate leaflet).
Olivary pretectal nucleus: pupillary light reflex
Ventral subparaventricular zone: vSPZ (*negative masking* in nocturnal animals)

And other target regions whose relation with ipRGCs is less well understood.

f) **Several cell types** (M1, M2, M3, etc. Fig. 10): roles poorly understood (see Do, 2019).

g) **Postnatal development**: Fig. 13 and comparison with SCN development.



Modello concettuale dell'orologio circadiano

Figura 10.1 Gli orologi circadiani dei mammiferi, di *Drosophila* e di *Neurospora* condividono la fondamentale proprietà di un circuito a *feedback* negativo. Concettualmente l'orologio circadiano consiste di due proteine, A e B, che formano un complesso proteico e che interagiscono grazie ai loro domini PAS. Questo complesso proteico attiva la trascrizione (regolazione positiva) che porta alla sintesi di una serie di proteine dell'orologio. Tali proteine forniscono poi il segnale di inibizione (regolazione negativa) che riduce o annulla l'induzione da parte delle proteine PAS (A e B). I fattori che determinano il periodo dell'oscillazione sono la stabilità delle proteine dell'orologio e la loro capacità di entrare nel nucleo e inibire il complesso delle proteine PAS. La stabilità di una proteina è spesso regolata dal suo livello di fosforilazione (P) da parte della proteina chinasi. Di solito la fosforilazione segnala che la proteina può essere distrutta. Tutti questi elementi fondamentali si ritrovano nei meccanismi dell'orologio dei mammiferi, di *Drosophila* e di *Neurospora*. In *Neurospora* le proteine PAS WC1/WC2 attivano la trascrizione (regolazione positiva), mentre la proteina FRQ assicura il segnale inibitorio. In *Drosophila*, l'induzione è fornita dal complesso proteico CYC/CLK (CLK è nota anche come JRK) e l'inibizione è assicurata dal complesso PER/TIM. Nei mammiferi, l'induzione proviene dal complesso BMAL1/CLOCK e l'inibizione dall'interazione fra le proteine PER e CRY.

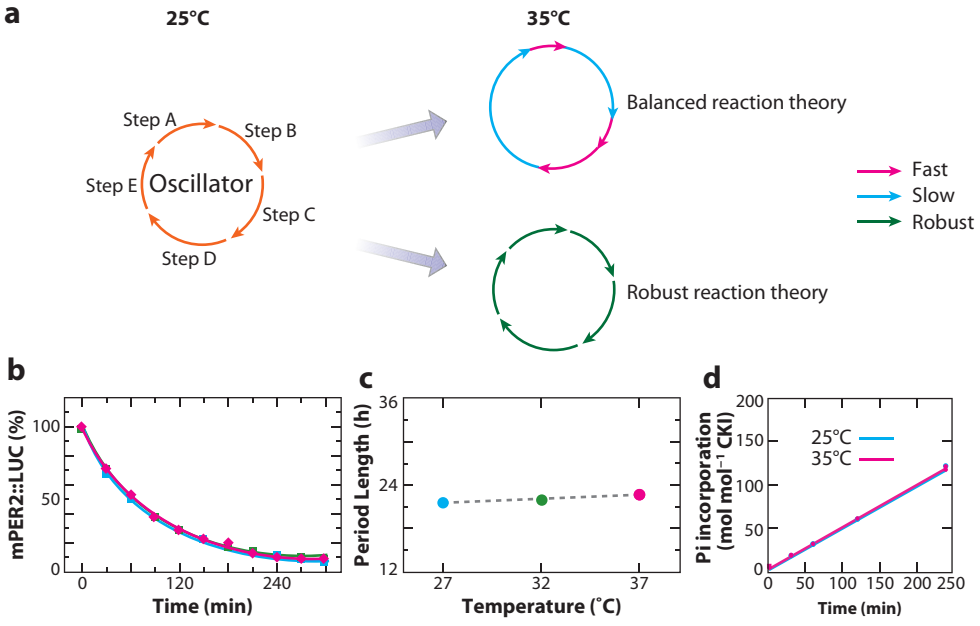
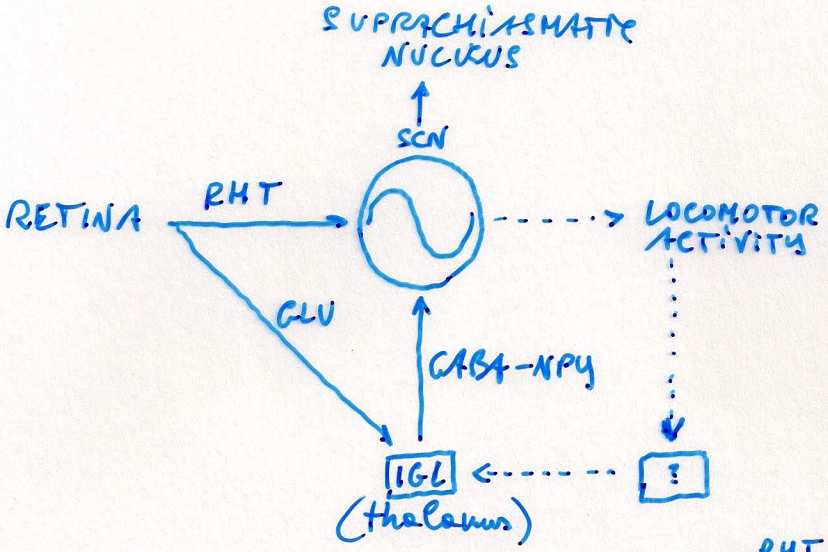


Figure 3

System analysis of clocks. (*a*) Two theoretical models of temperature compensation of the circadian clock. Steps A through E indicate the basic molecular reactions of circadian clock components. In the balanced reaction theory, increasing kinetic parameters of some basic reactions (*magenta*) shorten period, and those of other basic reactions (*blue*) lead to prolonging the period. These effects are offset so that the period is sustained. In the robust reaction model, such as the cyanobacterial circadian clock, temperature compensation of the circadian clock is caused by reactions of which kinetic parameters are independent (*green*). (*b*) The temperature dependency of decay for the mPER2::LUC bioluminescence in *mPer2*^{Luc} mouse embryonic fibroblasts (MEFs). The degradation of the mPER2::LUC protein was monitored after the addition of cycloheximide to MEFs. The time-course data of each sample were normalized to an approximate function in which time point zero was 100%. Each value represents the mean ± SEM (standard error of the mean) of the normalized data. The lines represent an approximated curve in which $y = 100$ at time zero and $y = 50$ at the averaged half-life time. The blue dots and line indicate the data at 27°C, green indicates 32°C, and magenta indicates 37°C ($N = 23$). (*c*) Temperature compensation in the period length of *mPer2*^{Luc} MEFs. The graph indicates the mean ± SEM. The gray broken line indicates the approximated line described by the equation $y = 19.02 + 0.097x$, and the Q_{10} value between 27°C and 37°C calculated from the equation is 0.957. (*d*) Temperature dependency of the ΔCKIε(wt) phosphorylation activity for the βTrCP-peptide substrate. Assays were performed at 25°C (*blue*) and 35°C (*magenta*). Abbreviations: ΔCKIε(wt), catalytic domain of wild-type (wt) casein kinase I epsilon (CKIε); Q_{10}, the change in the rate of a process caused by increasing the temperature by 10°C; βTrCP, β-transducin repeats–containing protein.

To examine the biochemical foundation underlying the observed temperature insensitivity, Isojima et al. (99) analyzed the phosphorylation activity of CKIε and CKIδ in vitro. They used synthetic peptide substrate derived from the putative β-transducin repeats–containing protein (βTrCP)-binding region of mouse PER2 and the catalytic domain of wild-type CKIε, lacking the C-terminal regulatory domain [ΔCKIε(wt)], to prevent the confusion that could result from the autophosphorylation of this regulatory domain and the subsequent repression of CKIε kinase activity. This use of the catalytic domain was also justified by evidence that CKIε is kept in a dephosphorylated, active state in vivo (105). Under this experimental condition, ΔCKIε(wt) phosphorylated the peptide substrate at similar rates both at 25°C

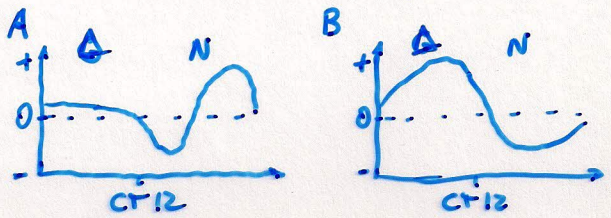


RHT = retino-hypothalamic tract

CT = CIRCADIAN TIME

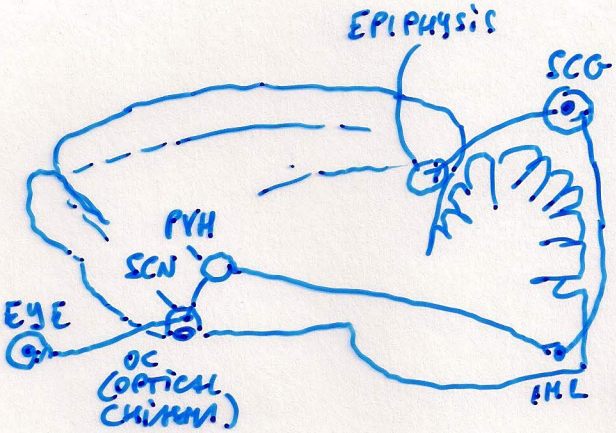
Δ-N = DAY and NIGHT (subjective)

IGL = intergeniculate leaflet (probably integrates the photoperiod signal with the motor activity)



phase response to light

EFFECT OF MOTOR ACTIVITY, INDEPENDENT OF LIGHT



(intermedio-lateral column thoracic segments of the spinal cord)

POSSIBLE MECHANISMS OF CONTROL OF PHOTOPERIODIC RESPONSE.

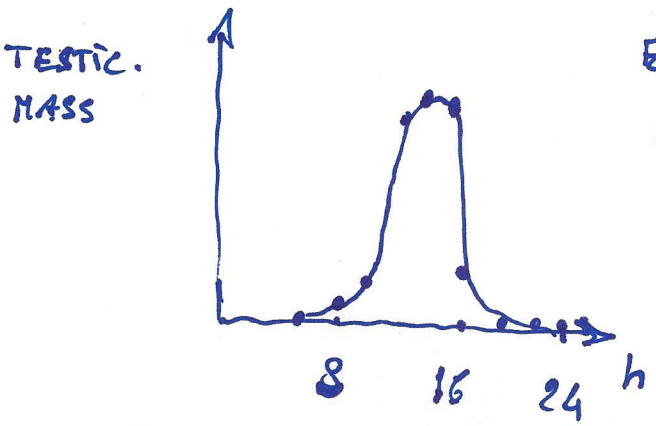
A. CLEPSYDRA THEORY

↳ ENOUGH LIGHT → THRESHOLD

B. CIRCADIAN CLOCK THEORY

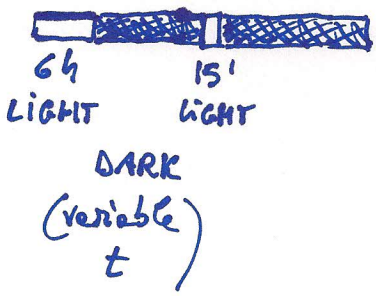
↳ STIMULUS IS EFFECTIVE ONLY DURING A CRITICAL PERIOD OF THE CYCLE

EXPERIMENTS IN JAPANESE QUAILS:



Effect of 15' light stimulus on testicle growth

for example:



TIMING OF STIMULI

Follett and Sharp, 1969

OTHER PHOTORECEPTORS

For instance,

BIRDS: photoperiodic synchronization also with covered eyes, especially with shaved skull.

PINEAL GLAND: close to brain surface in non-mammalian vertebrates. In some fishes there is a semi-transparent membrane.

It has cone-like photoreceptors in fishes, amphibians and reptiles.

Secretes **MELATONIN** (diffusible signal, cutting the neuronal fibers does not disrupts behavioral rhythms).

- A transplanted pineal gland has its own rhythm
- Pineal cells in culture display rhythmic secretion
- Cyclic melatonin injections synchronize the animal's activity

EYE + PINEAL (with SCN)

↓

RHYTHMIC MELATONIN SECRETION

The mechanism is conserved in vertebrates, but the relative importance of the pineal gland varies a lot, even among affine species (especially in birds).

For instance, removing the pineal gland has scarce effects in chicks or quails, whereas sparrows need SCN + pineal, which communicate via the **superior cervical ganglion** (sympathetic system); moreover the SCN receives informations from **deep encephalic fotoreceptors.**

In general, however, SCN is fundamental for rhythmicity (but not in *Danio rerio*, e.g.).

In mammals, photo-entrainment depends on the eyes.

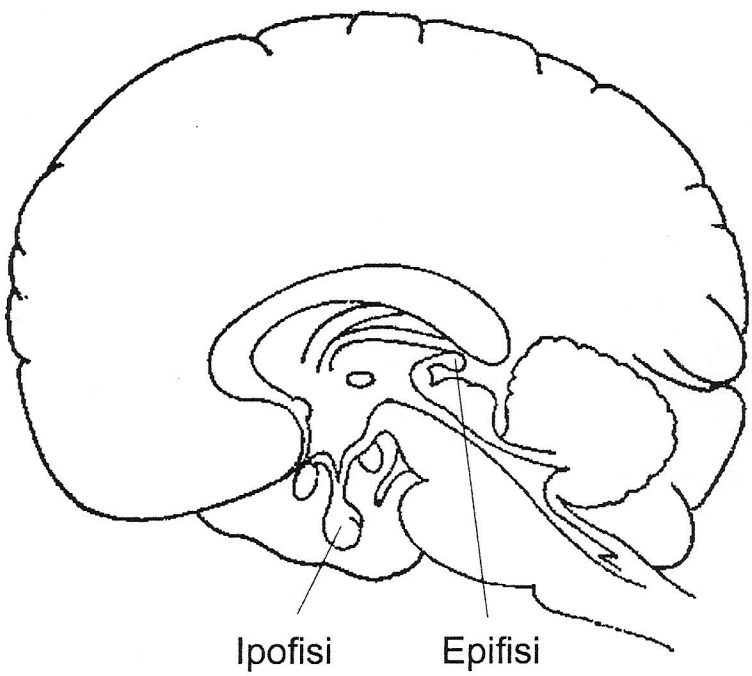


Fig. 1 - Sezione longitudinale del cervello per mostrare la posizione dell'epifisi e dell'ipofisi e i rapporti di queste ghiandole col terzo ventricolo.

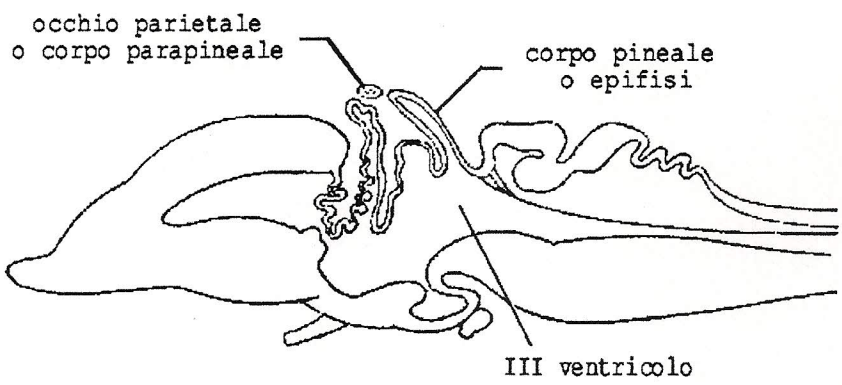


Fig. 2 - Posizione dell'epifisi e del corpo parapineale in un Vertebrato inferiore.

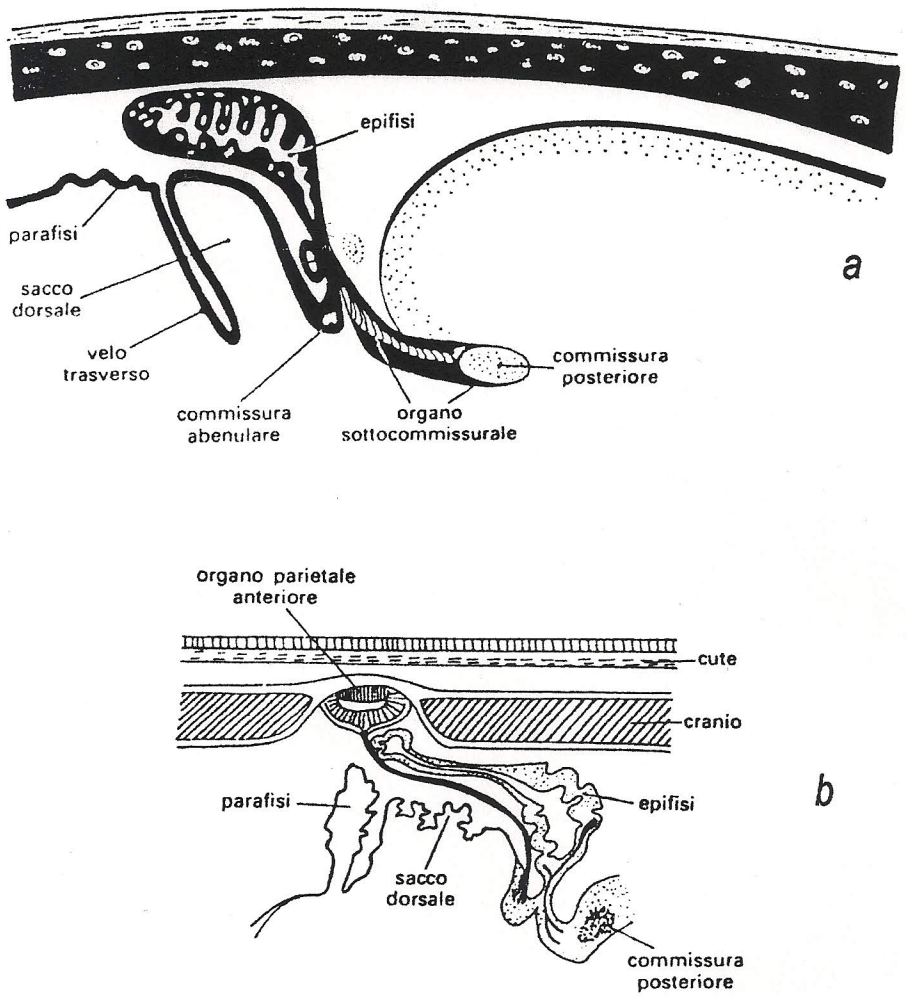
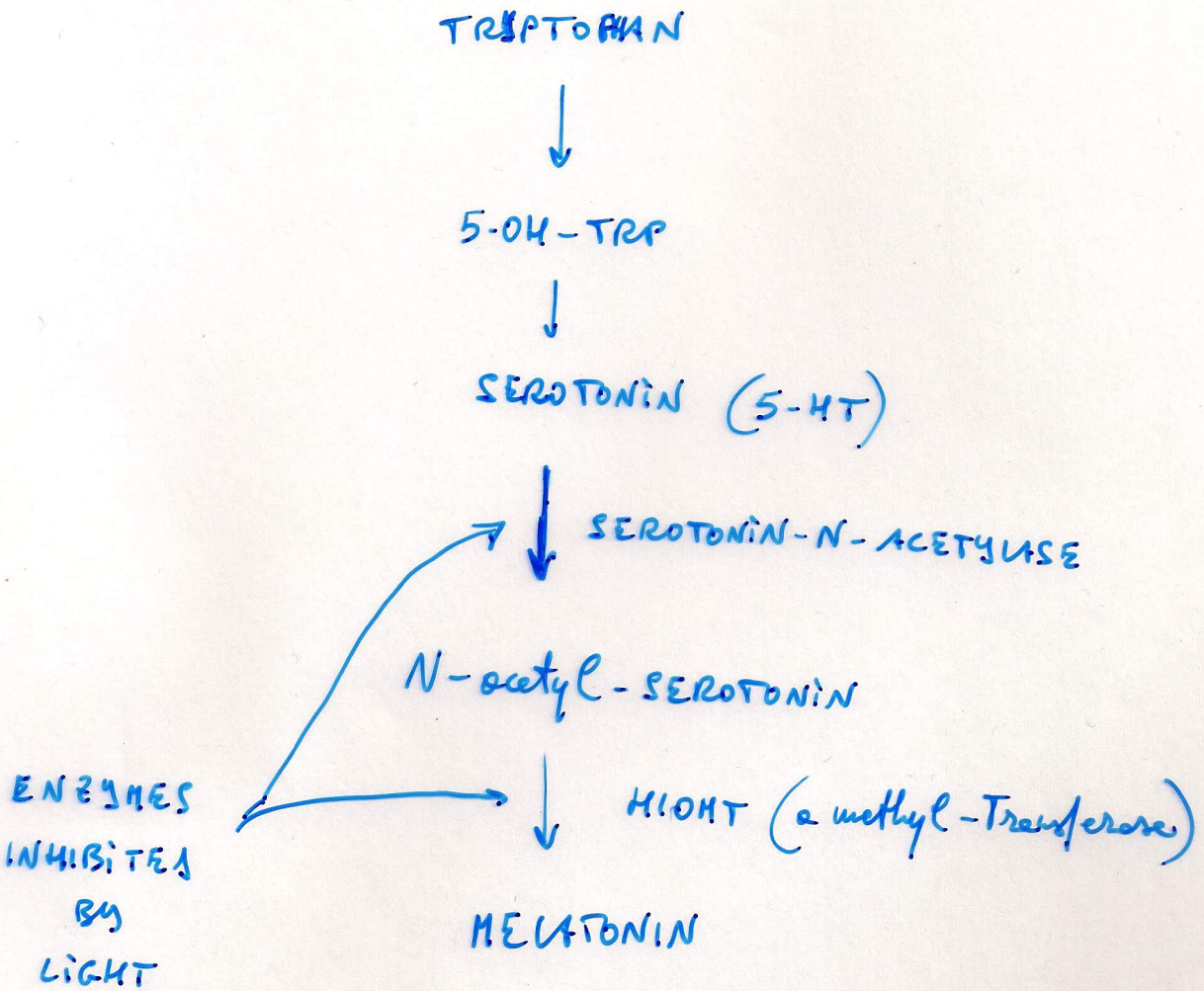
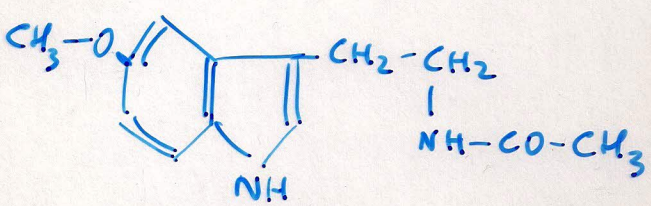


Fig. 10 - Sopra: epitalamo dei Teleostei. Sotto: organi parietali nell'epitalamo dei Sauri.

SYNTHESIS OF MELATONIN





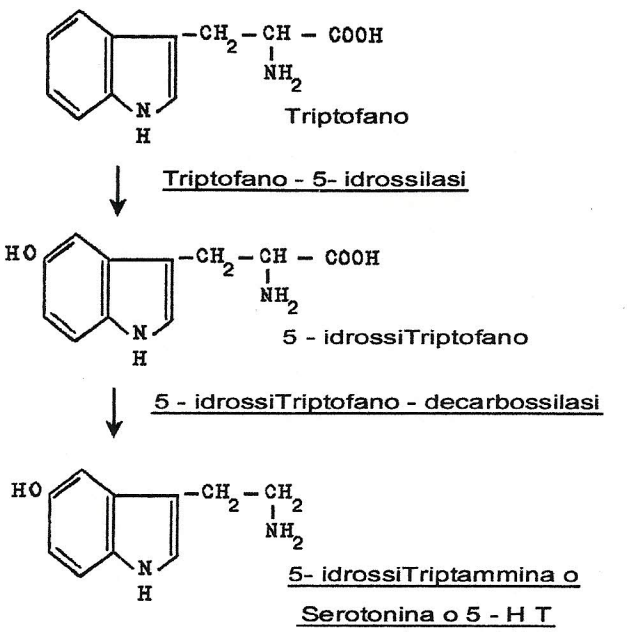


Fig. 15 - Formazione della serotonina dal triptofano mediante una reazione di idrossilazione e una di decarbossilazione. Le due reazioni sono catalizzate rispettivamente dalla triptofano-5-idrossilasi e dalla 5-idrossiltriptofano-decarbossilasi.

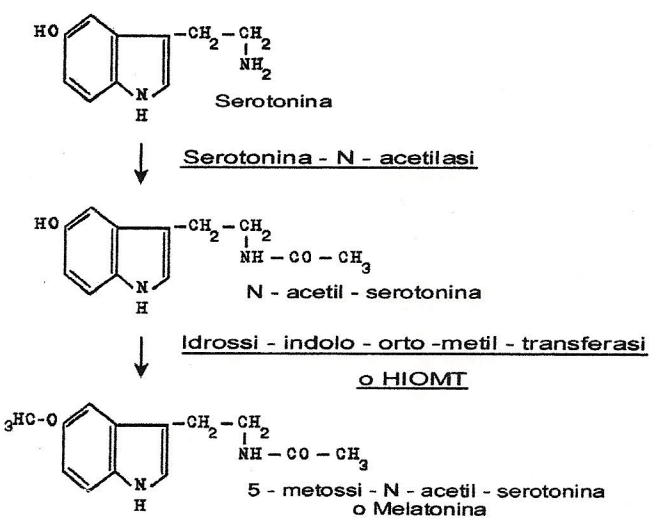
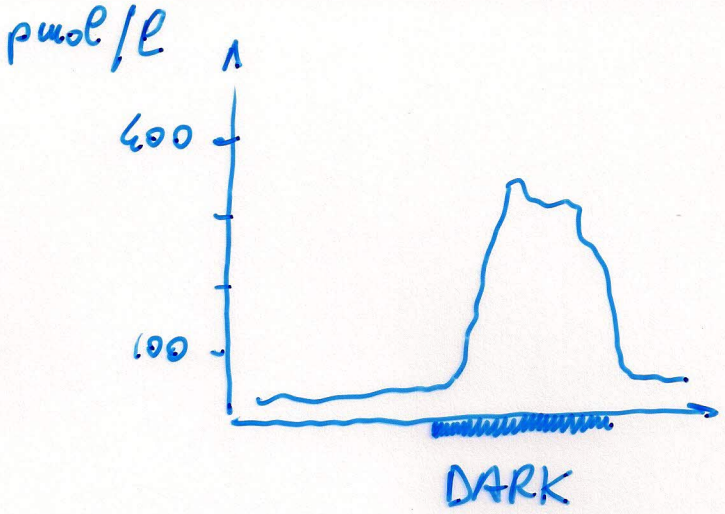


Fig. 16 - Formazione della melatonina dalla serotonina ad opera di due reazioni, una di acetilazione e una di transmetilazione. Queste reazioni sono catalizzate rispettivamente dalla serotonina-N-acetilasi e dall'idrossi-indolo-orto-metiltransferasi. Questi due enzimi sono inibiti dalla luce.

MELATONIN IN PLASMA



NIGHT :

SYMPATHETIC TERMINALS

↓

NE

↓

α_1 and β - ADRENERGIC (PINEALOCYTES)

↓

PLC / AC / I_{Ca}

↓

↑ Ca^{2+} and cAMP

↓

↑ MELATONIN SYNTHESIS

↑ ICER (inhibitor, represses the genes activated by ↑ cAMP and then blocks itself)

Therefore, the effect is dampened as time passes and finally the inducibility recovers

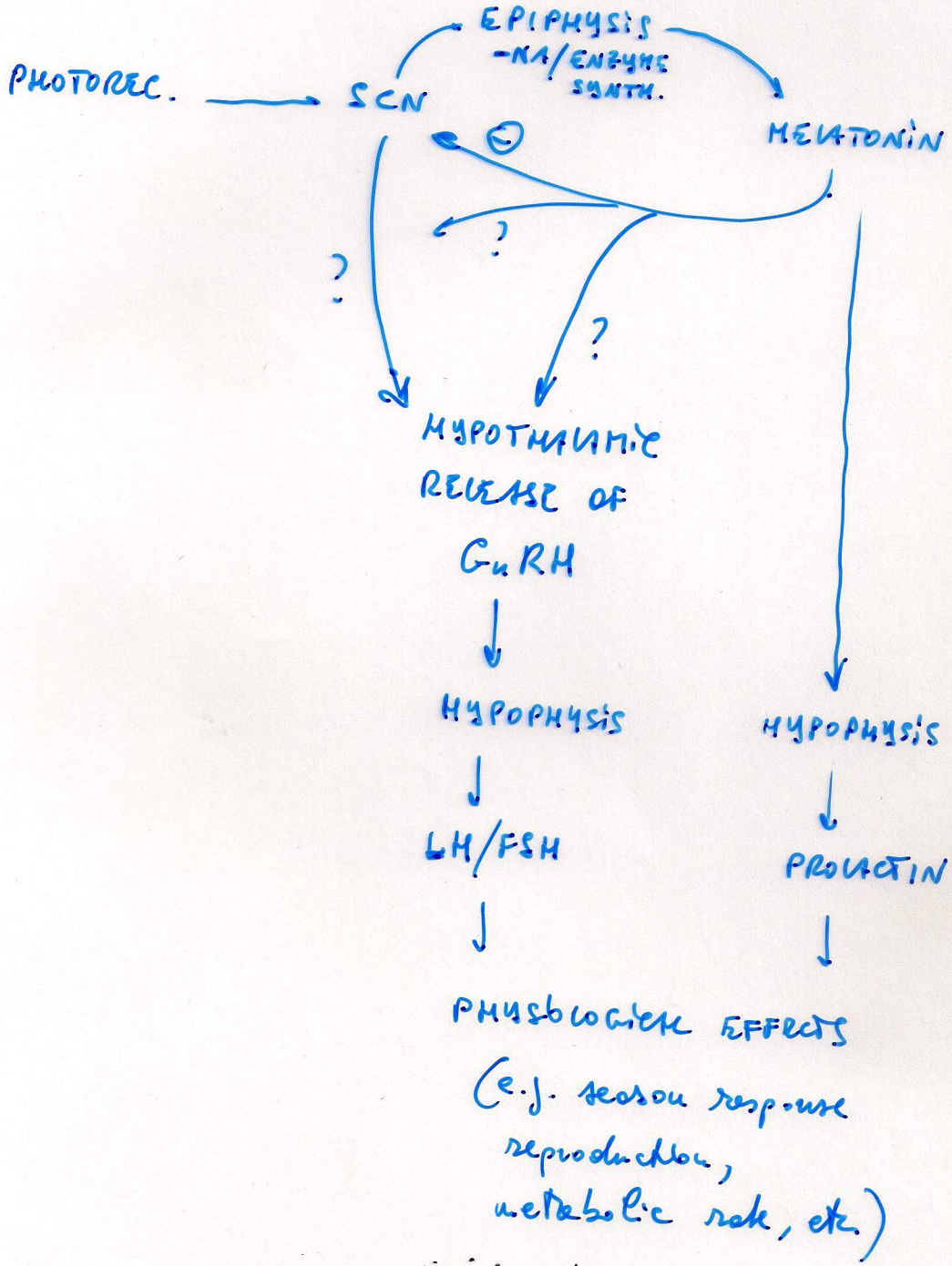
LIGHT : THE ADRENERGIC EFFECTS ARE ANTAGONIZED

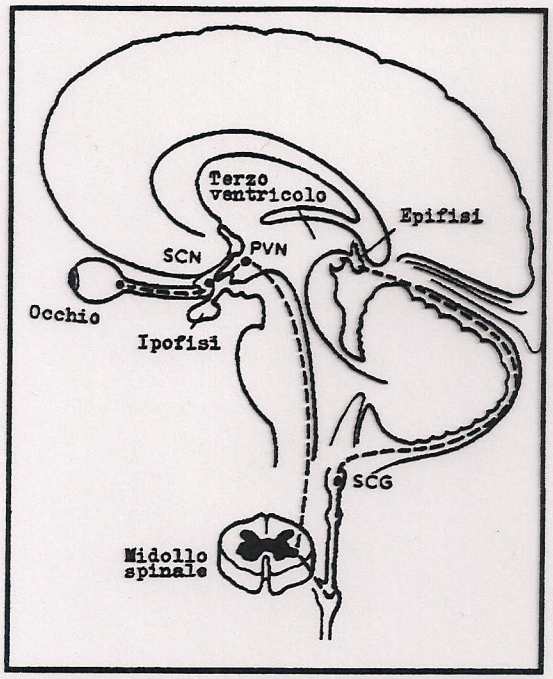
↓

↓ MELATONIN SYNTHESIS

MELATONIN RECEPTORS

- G PROTEIN COUPLED
- LOCALIZED IN : - SCN
  - medial thalamic nuclei
  - anterior hypophysis





Innervation by the diencefalic nuclei does not seem important for melatonin secretion.

Fig. 11 - Dai nuclei superiori del chiasma gli impulsi nervosi provenienti dalla retina arrivano alla ghiandola pineale tramite i nervi simpatici del ganglio cervicale superiore.

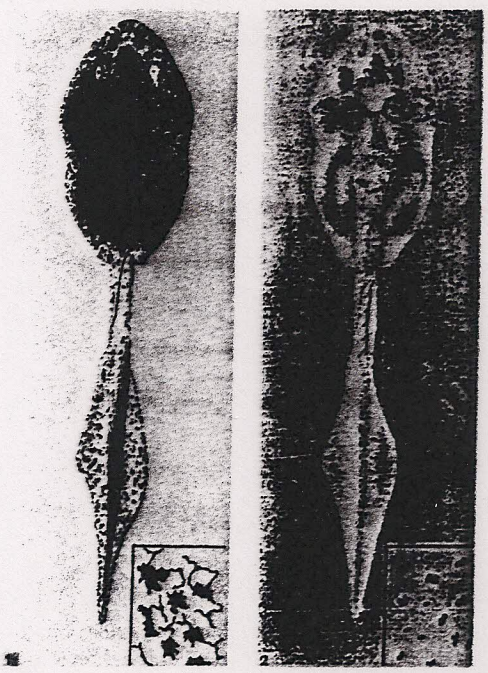
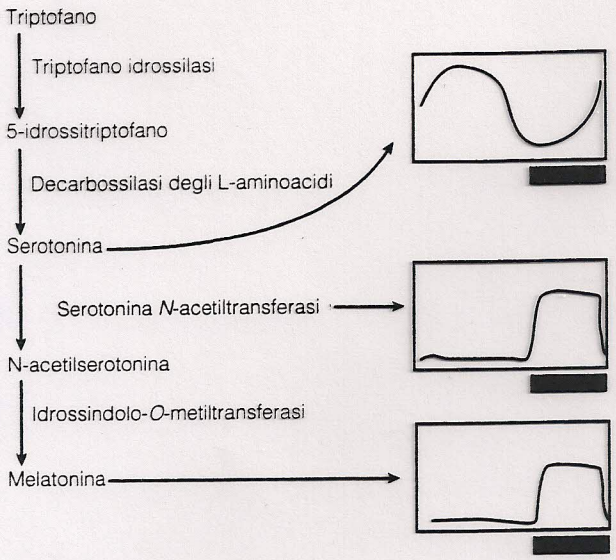


Fig. 12 - Un girino prima e 45 minuti dopo alimentazione con epifisi.



LUCE (LIGHT) ⊖ ⊖

FIGURA 45.11 Vie metaboliche epifisarie della produzione di melatonina a partire dal triptofano. A destra della figura sono rappresentati i ritmi circadiani di serotonina, serotonina *N*-acetiltransferasi e melatonina.

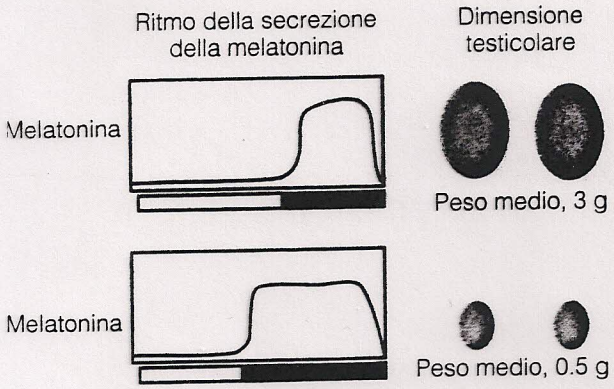


FIGURA 45.12 Ritmo della secrezione della melatonina e dimensione testicolare in criceti maschi in giorni con fotoperiodo differente. Quando si hanno 14 ore di luce e 10 di buio (LD 14:10), la durata della secrezione di melatonina è breve e i testicoli sono grandi. Invece, quando si hanno 10 ore di luce e 14 di buio (LD 10:14) la durata della secrezione di melatonina è lunga ed i testicoli sono piccoli.

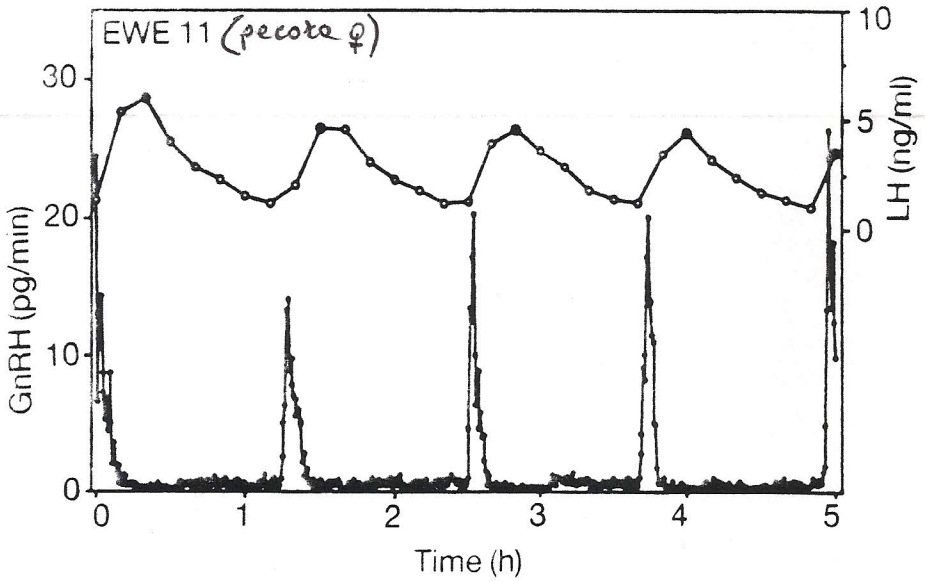
TESTICLE MASS

PRIMAVERA (SPRING)

AUTUNNO (AUTUMN)

Controllo inverso nella pecora, con riproduzione autunnale - invernale (Opposite regulation in the ewe)

HYPOPHYSIS DECODIFIES THE RHYTHM OF GnRH SECRETION, WHICH DEPENDS ON PHOTOPERIOD



SHORT DAY
↓
HIGH FREQUENCY OF GnRH SECRETION
↓
↑ LH
↓
↑ REPRODUCTIVE SYSTEM

Fig. 8.2. Rhythmic, pulsatile secretion of GnRH inducing the pulsatile release of the gonadotropic hormones LH and FSH. The data, obtained in the ewe by Moenter *et al.* (1992), show a frequency close to one pulse per hour; a similar frequency is observed in rhesus monkey and humans.

PULSATILE SECRETION: E.g. INSULIN

GLUCAGONE

GH

CORTISOL / ACTH-CRH

GnRH (LHRH)

(+) → LH
(+) → FSH

REFRACTORINESS OF REPRODUCTIVE SYSTEM:

To avoid multiple seasonal reproductive phases.

E.g., in the starling (Italian: storno), the reproductive system becomes refractory after 6 weeks (by a prolactin-thyroid interaction).

Subsequently, the system recovers when days are short (short photoperiod).

In general, **in birds**, the seasonal reproductive system does not depend on the pineal gland, i.e. on melatonin.

In **mammals**, refractoriness depends on the progressive insensitivity of the inhibitory effect of short days. In hamsters, pineal ablation produces reproductive activity independent of the photoperiod.

Human reproduction does not depend on photoperiod, although the anatomical and molecular components are present as in the other mammals and some primates have strong photoperiodic responses.

PHOTOPERIODIC SIGNALS IN BIRDS.

1) **Circadian system**:

LIGHT

↓ ↓ ↓

EYES Pineal gland BRAIN

↓ ↓ ↓ (variable importance)

PHOTOSYNCHRONY

2) **Seasonal photoperiodic system**: (studied with small optical fibers to stimulate small brain areas)

:

LIGHT

↓

ENCEPHALIC PHOTORECEPTORS

- hypothalamus
- area rostral to the hypothalamus

These cells contain a **photopigment** derived from opsin. Unclear interaction with SCN.

As in mammals: ↑GnRH → hypophysis → Hormones (LH/FSH) → Gonads

- REPRODUCTION
- SING (but here melatonin cooperates)

MIGRATION: other signals (i.e., not sex hormones).

Complex regulation by circadian rhythm and photoperiod.

For example, many birds become nocturnal during migration.