

ROLE OF MELATONIN IN TIMEKEEPING OF BIRDS

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SUMMARY

The pineal is a component of the avian timekeeping system. The best known output of the pineal gland is melatonin, a lipophilic molecule, whose presence appears to have been evolutionarily conserved. Two major physiological roles are assigned to melatonin. One role is its involvement in daily and seasonal timekeeping. The other is its involvement in the time measurement. Birds use daily rhythm of melatonin secretion to decode the time-of-day as well as the time-of-year information. Besides these two roles, the other physiological roles that melatonin performs include immune function, free radical scavenging, etc. Interestingly, in birds the pineal (melatonin) directly regulates several circadian behaviours, but not the circadian rhythm-mediated photoperiodic inductions; melatonin acts only as an endocrine modulator of the latter. In the present article the avian timekeeping system is very briefly described, and then the role of melatonin (and the pineal gland) in daily and seasonal timekeeping of birds is discussed.

Key words: Birds, melatonin, pineal gland, timekeeping.

INTRODUCTION

The molecule of melatonin (N-acetyl-5-methoxytryptamine) appears to have been evolutionarily conserved; many groups of animals and plants have been found containing the melatonin (1-4). In vertebrates, it is produced mainly by the pineal gland (1). However, extra-pineal sources (viz. lateral eyes, harderian gland, gut etc.) are also shown producing a small amplitude melatonin rhythm (2). Exogenously, light-dark (LD) cycle controls the time and duration of melatonin synthesis and secretion (for details and references see, 1, 2, 5-7). During day, amino acid tryptophan is taken up from the bloodstream and hydrolysed to 5-hydroxytryptophan (5-HTRP) in the presence of the enzyme tryptophan hydroxylase (TrH). Then, aromatic amino acid decarboxylase (AAADC) converts it into 5-hydroxytryptamine (5HT, serotonin). At night, arylalkylamine N-acetyltransferase (AANAT) acts on serotonin producing the N-acetylserotonin, which undergoes O-methylation by the enzymatic action of the hydroxyindole-O-methyltransferase (HIOMT). The final product of this biosynthetic pathway is the melatonin. The avian suprachiasmatic nucleus (SCN), the clock situated within the anterior hypothalamus overlying the optic chiasma (Fig. 1A) regulates the timing of melatonin synthesis at night. Neuronally, norepinephrine (NE) released from the sympathetic nervous system (SNS, Fig. 1A) regulates melatonin biosynthesis (1). Notably, the role of NE in birds appears different from what is known in mammals, in which norepinephrine activates melatonin production by pinealocytes during night. In birds, norepinephrine inhibits cAMP accumulation via alpha-2 adrenergic receptors and, in turn, inhibits AANAT activity and melatonin production during daytime (2).

Being a lipophilic molecule, all melatonin synthesized is believed to be released into circulation. Interestingly, however, at any given time the concentration of melatonin within the pineal is several magnitudes higher than that in the blood melatonin, indicating that a mechanism, hitherto unidentified, is additionally involved in release of melatonin. DNA microarray studies of chick pineal also show the presence of genes encoding a hormone-binding protein, the transthyretin(8), but it is yet to be investigated if such pineal proteins mediate the release of melatonin into circulation. In this review, I propose to briefly discuss the role of melatonin in daily and annual timing of behavioural and physiological events in birds. First, I will describe very briefly the avian timekeeping system, and then will discuss the roles melatonin (and the pineal gland) play in the timekeeping of birds.

TIMEKEEPING EVENTS

Similar to other organisms, birds possess endogenous timekeepers to regulate cyclicality (daily and annual) in their behaviour and physiology. These timekeepers are called "clocks" because of their precision in timing. They operate within the specialized cells, and are overtly expressed as rhythms in a myriad of functions. The frequency of these rhythms varies from minutes to months, but most frequently studied rhythms are those that are expressed in daily and seasonal events. The clocks operating on daily basis are called "circadian" (circa - about, dian - day; i.e. ~24-h period length), and those operating on annual basis are called "circannual" (circa - about; annum - year; i.e. ~1-year period length). Daily (circadian) events can be studied at multiple levels, but historically in vertebrate species, they have been studied mostly using the locomotion; e.g., perching activity in small birds

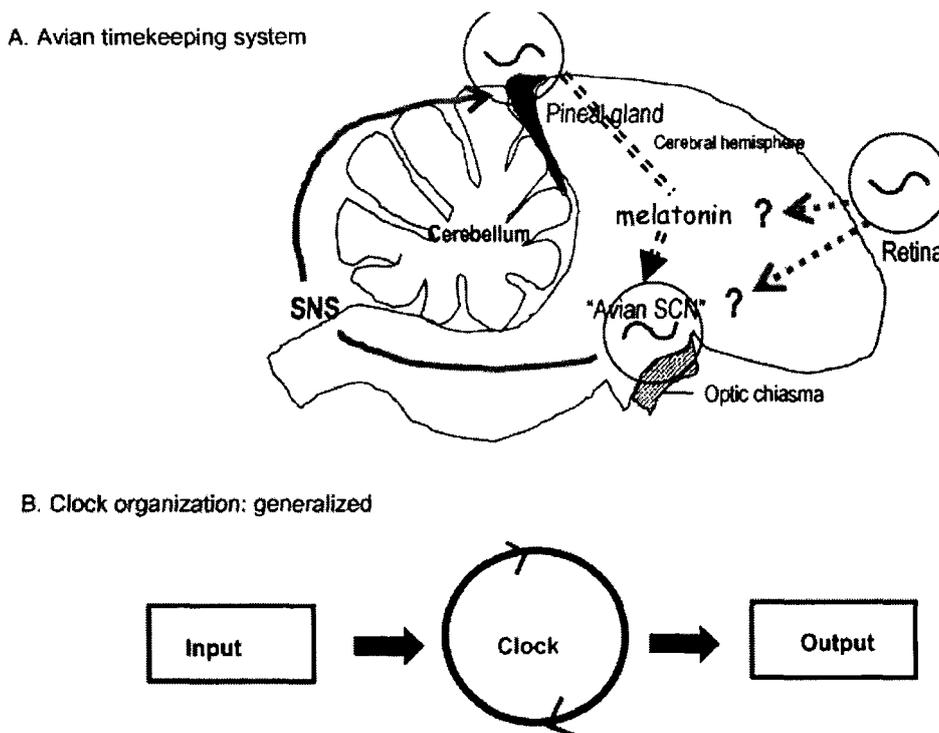


Fig.1. A: The circadian timekeeping system in birds comprises independent clocks at least at three levels: the pineal gland, the retina, and the hypothalamus. Melatonin is the best known candidate for the coupling between the pineal and hypothalamic clocks. Pineal melatonin biosynthesis is under the control of the hypothalamic clock (avian suprachiasmatic nucleus, SCN), via sympathetic nervous system (SNS), which releases norepinephrine (NE). In turn, melatonin synthesized by the pineal gland influences the activity of the avian SCN. How the retinal clock interacts with the other two clocks is still not understood. **B:** A generalized model of clock as conceptualized in a vertebrate species. It has an input, a clock and an output component.

and wheel running in rodents. On the other hand, the annual (circannual) events have usually been studied by reference to long-term (seasonal) events, such as migration, hibernation, reproduction, moulting, and so on.

AVIAN TIMEKEEPING SYSTEM

Intensive research has unraveled the mechanisms through which organisms generate circadian rhythms, yet it is not understood how circannual rhythms are generated and how are they integrated with other clocks in birds. Therefore, much of the account available on avian timekeeping system pertains to circadian (daily) timekeeping. A circadian timekeeping system is conceptualized as comprising three components: an input pathway, a central clock with an intrinsic capacity of generating precise oscillations, and an output pathway (Fig. 1B). In birds, the circadian timekeeping system is highly complex, as it comprises at least three independent central clocks (9) (Fig. 1A): the retinas of the lateral eyes, the pineal gland and the hypothalamus. Each of these clocks has its own input and output pathways. Thus, birds receive direct light input by the retinal cells, pineal cells as well as cells in the hypothalamus. Circadian clocks are present at all the three levels, and they all have indepen-

dent outputs. Nonetheless, all these clocks appear to interact with each other before producing the final output (10); melatonin is a known output of at least two of them, the pineal gland and the eyes.

PINEAL GLAND CONTAINS SELF-SUSTAINED CLOCK

Avian pineal is an organ with heterogeneous tissues. It consists of cells (pinealocytes), glia and lymphocytes, among other cell types (11). Recent microarray studies of chick pineal (8, 12) have revealed that the avian pineal houses hundreds of genes encoding multiple functions. There were at least 20 categories of gene transcripts recognized based on their known and unknown functions; the functional categories included genes involved in melatonin biosynthesis, circadian rhythm generation, photoreception/ phototransduction, immune and stress responses, binding to hormones, intermediary metabolism, protein modification, transcription factors, and miscellaneous functions (2).

In the late sixties, Gaston and Menaker (13) provided first evidence of avian pineal as a timekeeper from their experiments on house sparrow, *Passer domesticus*. When pineal was surgically removed (pinealectomy),

sparrows gradually lost circadian rhythmicity in their perching activity. In the following years, the effect of pinealectomy was studied in a number of bird species (for summary see 14). In general, pinealectomy had a dramatic effect on circadian rhythmicity in passeriform species, and not in galliform and columbiform species (for references see 14, 15). In species like Japanese quail (*Coturnix c. japonica*), pinealectomy had no effect (16), and in species like pigeons (*Columba livia*), it only disrupted and/or changed the circadian period (17, 18).

It is now established that avian pineal contains independent input-pacemaker-output system required to perform its role as a timekeeper (9, 12, 14). Each pinealocyte contains a circadian oscillator, since circadian rhythmicity in melatonin production continues over several days under constant darkness (DD) or dim red light (LL_{dim}) in both organ and dispersed cell culture (19, 20). The input component comprises several opsin-based photoreceptive molecules [e.g. pinopsin (21, 22), melanopsin (20, 23), photoisomerases, gRh and gRgr (24)] and signal transduction mechanisms (25). Molecular studies of avian pineal report the presence of all those clock genes, which are implicated in circadian rhythm generation within the SCN of rodents. Chick pineal is shown expressing clock, bmal1 and bmal2, cry1 and cry2, per2, per3, E4bp4 (bZIP transcription factor) (26-33). The first transcriptional profile of a bird (chick) pineal, using cDNA microarrays, revealed that 382 genes in LD and 128 genes in DD were expressed rhythmically with at least a two-fold change (8). Many of these genes were also rhythmic in dispersed pineal cell culture, albeit with a relatively lower amplitude (Karaganis, Kumar et al., unpublished). Melatonin is the best-known circadian output of the pineal cells. How the activity of the core circadian oscillator(s) of pinealocytes is linked with multi-step enzymatic process-mediated melatonin biosynthetic machinery remains largely unknown.

MELATONIN RHYTHM ENCODES DAY AND SEASONS

The duration of nighttime melatonin production reflects the night length and, hence, the day length (34, 35) (Fig. 2A). This suggests that melatonin rhythm provides a calendar to birds based on the photoperiodic information of the environment. Importantly, the pineal can recall in vitro the photoperiodic information it has acquired in vivo. In an elegant series of experiments, Brandstätter and his colleagues (35) provided evidence that the explanted pineals from house sparrows maintained under short days (8L:16D) and long days (16L:8D) had continued short and long day patterns of melatonin secretion for several days. This meant that the pineal gland stored and retained the photoperiodic information. The capacity of the pineal gland in storing the biologically meaningful information about time may enable birds to compare and measure the day length, as season progresses, and to

buffer the effects of adverse environmental conditions; for example when the photoperiodic measurement may be tampered temporarily due to unfavorable periods, like weather storms etc.

The amplitude of melatonin varies seasonally (Fig. 2B) and, hence, can provide the annual information to birds (36). In the house sparrow, for example, melatonin levels are high in summer, low in winter, and intermediate in spring (36). Similarly, the amplitude of plasma melatonin rhythm is significantly reduced during migratory seasons in the migratory garden warbler *Sylvia borin* (37), and during summer months in the Antarctic penguin *Pygoscelis adeliae* (38, 39) and the Arctic svalbard ptarmigan *Lagopus mutus hyperboreus* (40).

DAILY AND SEASONAL TIMEKEEPING: ROLE OF PINEAL GLAND AND MELATONIN

Daily (circadian) timekeeping

In birds, the effects of melatonin appear in contrast to that of its effect in mammals. For example, melatonin is critical for normal circadian rhythmicity in birds, but it has at the most a modest modulatory effect on the circadian functions in mammals. The converse is true of the effects on photoperiod-induced seasonal breeding; melatonin is critical in mammals but not in birds (for references, see 14).

Melatonin performs two important roles the relative importance of which may be species-specific. (1) Melatonin is a potential clock component. The retinal and pineal clocks exert their circadian effects *via* their melatonin rhythms. Therefore, the absence of melatonin rhythm leads to arrhythmicity. (2) Melatonin acts as a coupling agent within the timekeeping system of birds. It affects either the frequency of one or more oscillators or the strength of coupling among different oscillators, or both (9, 41, 42). Any change in the melatonin profile can, therefore, induce changes in the circadian system functions. For example, a high-amplitude melatonin will strengthen the mutual coupling between component oscillators and, hence, the net result will be enhancement of the self-sustainment of clock. Consequently, the susceptibility of the circadian system to photoperiodic noise will be decreased. In a similar way, reduced melatonin amplitude will weaken the mutual coupling between component oscillators and, hence, the net result will be attenuation of the self-sustainment of the clock. This, in turn, will facilitate the adjustment of the circadian system to changing Zeitgeber conditions.

Most studies in birds have measured the effects of pineal / melatonin on circadian behavioural rhythms; e.g., locomotion and feeding activities. Earlier studies showed that the pinealectomy, and not the neural disconnection of the pineal gland or chemical sympathectomy, disrupted circadian rhythmicity in house sparrows (13, 42-

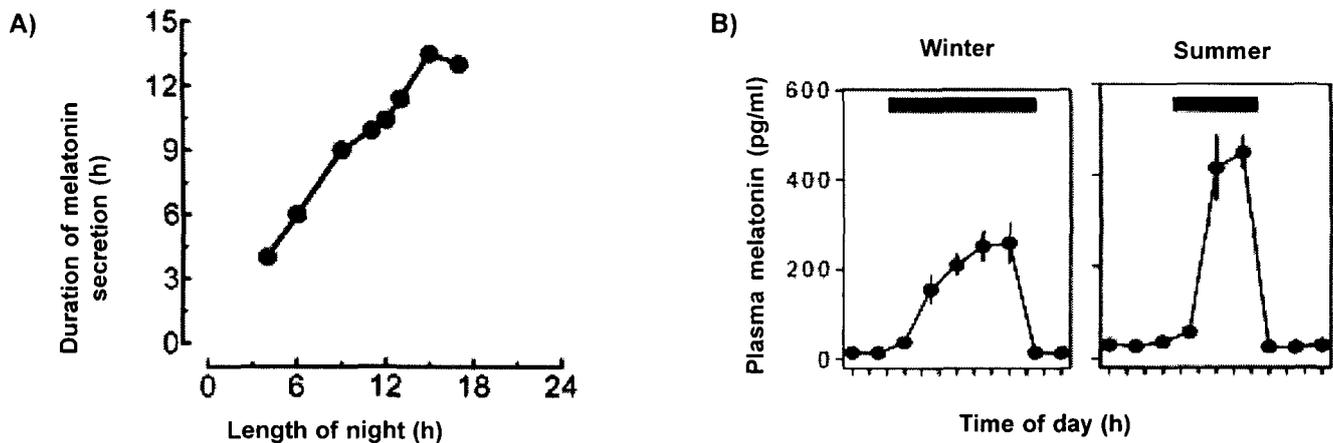


Fig.2. Changes in the melatonin profile as function of day length and season. **A:** Relationship of melatonin secretion with night length as determined in the Japanese quail (28). Approximately, the duration of melatonin peak increases by 0.8 h per hour increase in the duration of night. **B:** Changes in the duration and amplitude of plasma melatonin secretion in the house sparrow during winter and summer months (30).

44). Pinealectomized arrhythmic sparrows became re-rhythmic when the pineal gland from another sparrow was transplanted in their eyes (45). This led to the conclusion that a humoral substance (in all probability, melatonin) was involved. The involvement of melatonin in regulation of circadian behavioural rhythms was further confirmed by several subsequent studies. Periodically applied exogenous melatonin by infusion or through drinking water restored rhythmicity in arrhythmic pinealectomized sparrows and pigeons (17, 46). In a recent study, we investigated the effects of pineal removal on circadian activity rhythms in the Indian weaver bird (*Ploceus philippinus*). In weaver birds entrained to 12L:12D, pineal removal led to gradual loss of circadian rhythmicity (Fig. 3), similar to that reported in the house sparrow (for references, see 37).

A few other studies have examined the role of melatonin in the regulation of circadian rhythms other than those involved in the regulation of locomotion and feeding activities. In house sparrows, pinealectomy caused arrhythmicity in 2-deoxy [14 C] glucose (2-DG) uptake by the visual system (47), and daily melatonin administration restored rhythmicity in 2-DG uptake in these arrhythmic individuals (48). McGoogan and Cassone (49), from their study in chicken, suggested that circadian rhythmicity of retinal visual function, recorded by electroretinogram (ERG), was regulated at least partially through melatonin. Melatonin injections also induce an advance phase shift in the corneal mitotic rhythm in quail kept under 12L:12D, but not under LL when endogenous melatonin is suppressed (50), suggesting that melatonin is involved in phasing, but not generation, of the rhythm.

Overall, the role of melatonin in avian circadian rhythmicity is not uniform. In some species (e.g., house sparrow) melatonin influences circadian rhythmicity, but in others, like Japanese quail, melatonin-independent circadian rhythms also occur. There are still other species (e.g., European starling), which show an intermediate response to pinealectomy. Changes and differences in specific features of melatonin signal can also be found to vary under a variety of natural environmental conditions. For example, pinealectomy abolished circadian rhythmicity in house sparrows when they were kept under constant conditions and free-run, but not when they were kept under LD and synchronized. Furthermore, there was always a residual rhythmicity for-a-while in pinealectomized house sparrows (13, 42). On the other hand, lesions of the hypothalamic clock resulted in the severe impairment of circadian rhythmicity in house sparrows although they still had their pineals intact (51, 52). All these meant that melatonin interacted with at least one oscillator other than from which it is produced to regulate circadian rhythms in birds. In all probability, melatonin acts on the avian SCN. In the absence of melatonin signal, SCN functions as a damped oscillator. It does function as a self-sustained pacemaker in the absence of melatonin signal, however, if there was a direct access to the photic input. This, perhaps, would explain why pinealectomized sparrows retain circadian rhythmicity under LD cycle.

Seasonal (circannual) timekeeping

Birds measure day length using their circadian system (10). Since melatonin is the part of the circadian system of birds (2, 10, 14), melatonin may be assumed to be involved in regulation of the photoperiodic effects

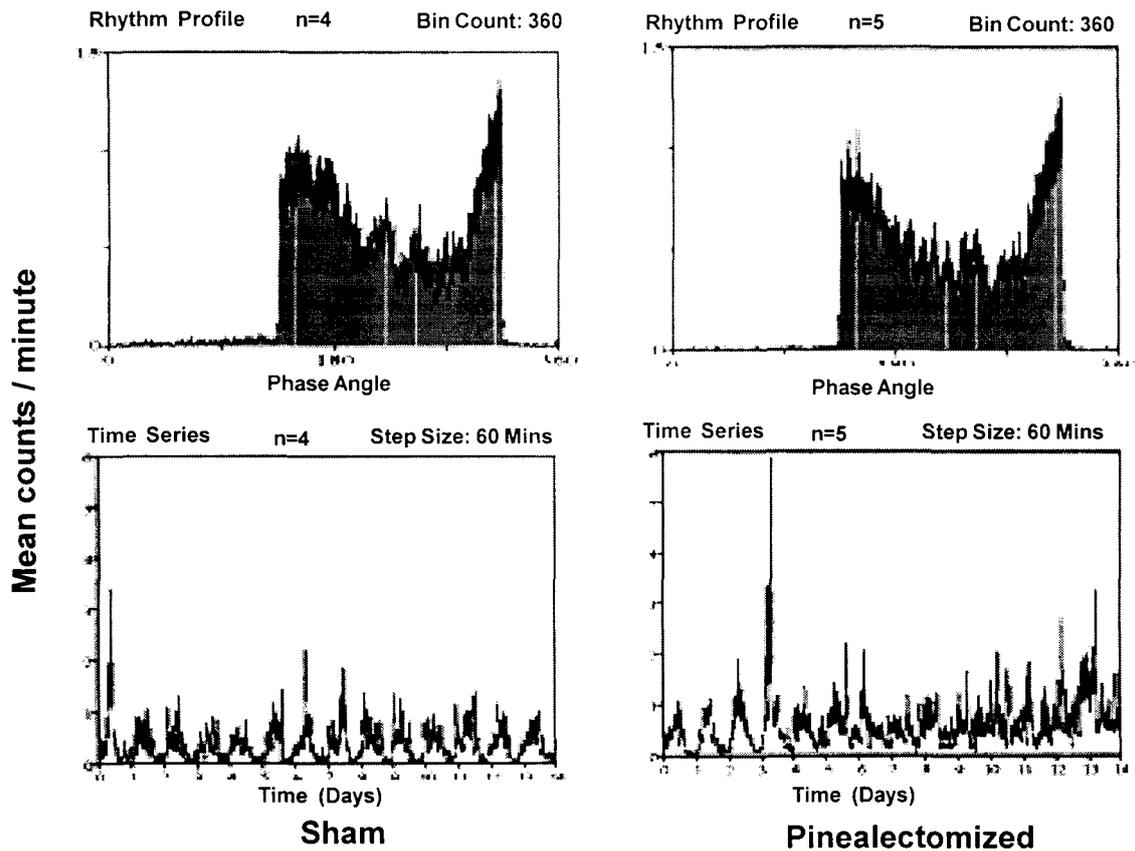


Fig.3. Effect of surgical removal of the pineal gland on circadian locomotor activity in male Indian weaver birds exposed first to 12L:12D and then to constant dim light (LL_{dim}). Upper panels show the average pattern of daily activity in both the groups before the operation. Lower panels show oscillations in activity bouts from the day birds of both groups were released into LL_{dim} . Note the gradual loss of circadian rhythmicity in pinealectomized (right hand lower panel) but not in sham (left hand lower panel) birds, especially during the period cycle 7 onwards. Sham – operated but pineal not removed; pinx – pineal surgically removed.

at one level or the other (Fig. 4). However, melatonin signal appears to be redundant as far as photoperiodic effects on gonadal growth and development in many bird species including the European starling *Sturnus vulgaris* (53), spotted munia *Lonchura punctulata* (54), the tree sparrow *Spizella arborea* (55), the Japanese quail *C. c. japonica* (34), the black-headed bunting *Emberiza melanocephala* (56) and the red-headed bunting *Emberiza bruniceps* (57) are concerned. The absence of a role for melatonin in photoperiodism could mean that the deep brain photoreceptors (DBPs), which are necessary and sufficient for the detection of changes in day length that regulates avian reproduction, are not linked to the reproductive axis via the circadian system. The effects of pinealectomy or melatonin injections are shown, none the less, in some birds, including the domestic duck *Anas platyrhynchos* (58), the Indian weaver bird *Ploceus philippinus* (59; also see 57 reporting the absence of melatonin effects in certain phases of gonadal growth and development cycle), the Indian jungle bush quail *Perdica asiatica* (60), rose-ringed parakeet *Psittacula krameri* (61)

and the lal munia *Estrilda amandava* (62). In our recent experiments, we have further shown melatonin as modulator of testicular growth and development in buntings. In one of the studies, we implanted subcutaneously melatonin-filled or empty silastic capsules in black-headed buntings that were subjected to 11.75 h light per day (11.75L:12.25D) under red or white light. The buntings that had melatonin capsules and were exposed to red light had significantly larger testes (Fig. 5). These results are consistent with the hypothesis that elimination of melatonin rhythm probably alters the perception of day length in photoperiodic species. In another study (63), we investigated the modulatory role of melatonin in the endocrinology of photoperiodic induction of testicular growth in the red-headed bunting. In buntings, exogenous injections of prolactin attenuate the induction of a photoperiodic response under long days (64). But, Trivedi et al (63) showed that pretreatment with melatonin blocked the suppressive effects of prolactin on photoperiodic induction of testicular growth and development in the red-headed bunting.

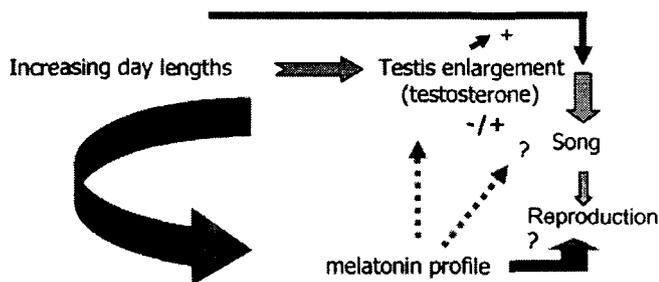


Fig.4. Hypothetical scheme of the possible involvement of melatonin in photoperiodically regulated seasonal events in birds. Melatonin can influence seasonality at one or more than one levels. As conventional (+) sign is used for the stimulation and (-) sign is used for the inhibition of the photoperiodic effects. (?) sign is used to indicate controversial reports.

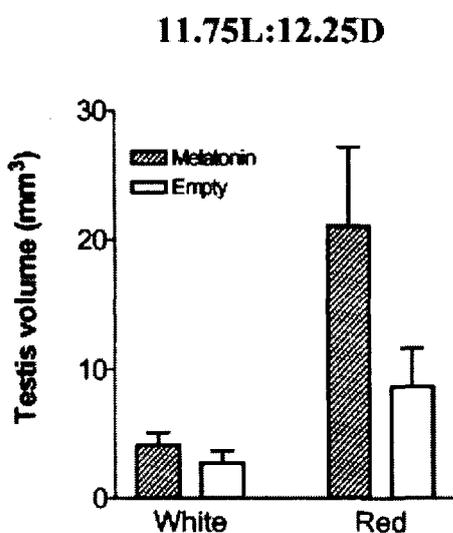


Fig.5. Effect of melatonin on modulation of the photoperiodic induction. Black-headed buntings were implanted with melatonin-filled or empty silastic capsule's and exposed for 8 weeks to 11.75L: 12.25D with light phase in red (long light wavelength) or white light. Note the acceleration of photoperiodic induction of testis volume in bird exposed to red light.

CONCLUSION AND PERSPECTIVE

Birds possess a highly complex and diverse, but rather a plastic, timekeeping system, which comprises at least three independent central clocks, in the eyes, in the pineal and in the hypothalamus. All the three clocks interact with each other, although their degree of interdependence varies from species to species. In the whole scheme of timekeeping in birds, which centers on evolving a successful temporal strategy in the geophysical environment, melatonin appears to be the key component. In the absence of melatonin, even the hypothalamic

clock may not sustain its high-amplitude circadian oscillations. We have the reports on daily melatonin profiles and the results from removal / replacement type of studies (removal of pineal gland and exogenous administration) from a number of bird species, yet we are far away from understanding how melatonin meets the demands of a multi-oscillatory avian timekeeping system. Moreover, whether results from the laboratory experiments will hold good on free living birds is another dimension of research which needs to be pursued.

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