Melatonin: An internal signal for daily and seasonal timing

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Melatonin is secreted only during night, irrespective of the habitat of an organism and the site of its synthesis and secretion, and hence known as “darkness hormone”. Elevated melatonin levels reflect the nighttime. In vertebrates, the main site of melatonin production is the pineal gland. Species in which melatonin is also secreted from sources other than the pineal, as in some birds, relative contributions of different melatonin producing tissues to the blood melatonin level can vary from species to species. Melatonin acts through its receptors, which are members of the G protein-coupled (GPCR) superfamily. Three melatonin receptors subtypes MT1 (mel1a), MT2 (mel1b), and MT3 (mel1c) have been identified in different brain areas and other body organs of vertebrates. Melatonin synthesis and secretion are circadianly rhythmic. Changes and differences in specific features of melatonin signal can vary among species, and under a variety of natural environmental conditions. Two major physiological roles of melatonin are established in vertebrates. First, melatonin is involved in the circadian system regulated behavioural and physiological functions. Second, it is critical for the photoperiodic system. Besides, melatonin has been implicated in various ways both directly and indirectly to human health, including jet lag, sleep, immune system and cancer.

Keywords: Bird, Clock, Melatonin, Photoperiod, Sleep

As in other vertebrates, especially in mammals, the main site of melatonin production is the pineal gland, which in most species is situated within the recess formed by the two cerebral hemispheres and the cerebellum. Each pineal cell (pinealocyte) synthesizes and secretes melatonin, as evidenced by in vitro experiments. Cultured pineal cells exhibit significant rhythm in the melatonin production, similar to that exhibited by the pineal gland. Several reports show significant amount of melatonin production by retinae of the lateral eyes, harderian gland, and gastrointestinal tract. In principle, circulating melatonin levels faithfully reflect total melatonin production by an organism. Relative contributions of different melatonin producing tissues to the blood melatonin level can vary from species to species. In this aspect, the roles of the eyes and the pineal have been investigated in a few species. In the House sparrows (Passer domesticus) most, if not all, blood melatonin is secreted by the pineal gland; surgical removal of pineal gland renders sparrows with undetectable levels of melatonin. In Japanese quail (Coturnix coturnix japonica) and pigeon (Columba livia), on the other hand, only a little over than half of blood melatonin comes from the pineal gland; the remaining, a little less than half of blood melatonin, comes from the retinae and other undefined tissues.

Melatonin acts through its receptors, which are members of the G protein-coupled receptor (GPCR)
superfamily. Three melatonin receptors subtypes have been identified in vertebrates: MT1 (mel1a), MT2 (mel1b), and MT3 (mel1c). MT1 and MT2 have been identified in all vertebrates, which have been investigated, but MT3 has been found only in non-mammalian species. These receptors have been identified in different brain areas and other body organs. In mammals, a high density of melatonin receptors has been found in the suprachiasmatic nuclei (SCN), which is the principal site of the biological clock in vertebrates and pars tuberalis (PT) of the anterior pituitary, which is involved in the photoperiodic regulation of reproduction in both birds and mammals.

In mammals, there is a peculiar orphan melatonin-related receptor (GPR50), to which melatonin does not bind under in vitro expression assays and remains a puzzle from a functional perspective. Recently, by analysis of Mel1c synteny, GPR50 is shown to be the mammalian orthologue of Mel1c. Levoye et al. believed that GPR50 acts as a dimerization partner for mammalian melatonin receptors, thereby modulates melatonin signal transduction in the ligand-independent manner. A difficulty with this proposition is rather limited overlap between melatonin receptor and GPR50 expression, the former being concentrated in the pars tuberalis (PT) and the latter showing a strongest expression in the brain where melatonin receptor expression is weak or absent. In the hypothalamus, GPR50 is expressed in areas linked with energy homeostasis [e.g. dorsomedial hypothalamus (DMH) and paraventricular nucleus (PVN)]. Intriguingly, GPR50 in rodents is also expressed in the ependymal cell layer lining the third ventricle, and thus overlaps with distribution of Dio2 and Dio3 mRNA expression. In Siberian hamsters (Phodopus sungorus), exposure to short photoperiods leads to a loss of body fat as well as a reproductive switch off, along with reduced GPR50 expression levels in the MBH. GPR50 knockdout mice show a lean phenotype, with an unusual tendency to express torpor in response to food restriction. Taken together the results from several findings, GPR50 appears to be linked with the metabolic regulation, rather than directly to the photoperiodic response, which could be important in finding insights into its ligand search.

The literature on pineal and melatonin is vast, and so it may not be possible to review it in a single review, like the present one. Therefore, because of our own bias and limitation, the present review is restricted mainly to birds with relevant information on mammals, for the sake of comparison, with focus on the role of melatonin in circadian and seasonal events in birds.

**Regulation of melatonin synthesis and secretion**

The regulation of melatonin synthesis and secretion has been studied in the avian pineal gland. Several studies have established that day-night cycle of the environment controls the time and duration of melatonin synthesis and secretion. Briefly, during the day, amino acid tryptophan is taken up from the bloodstream and hydrolyzed to 5-hydroxytryptophan (5-HTP) in presence of the enzyme tryptophan hydroxylase (TRH). Then, it is converted to 5-hydroxytryptamine (5HT, serotonin) by the action of the enzyme, aromatic amino acid decarboxylase (AAADC). During the night, serotonin is acted upon by the enzyme arylalkylamine N-acetyltransferase (AANAT) forming N-acetylserotonin. The latter undergoes O-methylation by the action of the enzyme hydroxyindole-O-methyltransferase (HIOMT) producing melatonin, the final product of the biosynthesis. All melatonin synthesized is released into the circulation since it is a lipophilic molecule. But at any given time, the concentration of melatonin within the pineal gland is several folds higher than that of the blood suggesting that there may be some mechanism, hitherto unidentified, involved in the release of melatonin. Microarray studies of the pineal gland show the presence of genes encoding hormone-binding proteins, like transthyretin. Neuronally, norepinephrine is shown to be involved in the regulation of melatonin biosynthesis but its role appears to be different in birds than what is known from the biosynthesis of melatonin from the mammalian pineal gland. In birds, norepinephrine inhibits cAMP accumulation via alpha-2 adrenergic receptors and, in turn, inhibits AANAT activity and melatonin production during daytime. By contrast, in mammals norepinephrine activates melatonin production by the pinealocytes during night.

**Melatonin secretion is a circadian rhythm**—Melatonin is produced rhythmically; circulating levels are low during the day and high during the night. The rhythmicity continues both in vivo and in vitro under constant conditions of darkness (DD) or dim light (LL) with a period close to 24 h. Further, an advance or a delay of the Zeitgeber (zeit = time; geber = giver) conditions (e.g.
LD-cycle) produces corresponding phase shifts of the melatonin rhythm, and this phase shift is seen even when the animal is subjected to constant conditions, suggesting that the underlying circadian melatonin oscillator was entrained\(^{30,31}\). A further confirmation that the properties of the melatonin rhythm are consistent with the melatonin oscillator comes from T-experiments. Exposure to T-photocycles (LD cycle with varying periods, e.g. 22, 24, 26 h etc.) produces changes in the phase of melatonin rhythm\(^{27,32}\) as one would predict from the oscillatory theory. This is not surprising as the biochemical pathway involved in the synthesis of melatonin appears to be regulated by the circadian clock, which resides within each pinealocyte. This clock is clearly seen operating both at the transcriptional level (as seen in the rhythmic expression of mRNAs of TRH, AANAT and HIOMT) and posttranscriptional level (AANAT protein activity parallels the presence and absence of AANAT mRNA expression)\(^{4,33,34}\). At molecular level, pinealocytes appear to be equipped with all those genes, which are implicated in the generation of circadian rhythmicity in other tissues including the hypothalamic clock. However, the degree of persistence of circadian rhythmicity could vary between species\(^{28,30,31,35,36}\).

**Role of melatonin in the regulation of physiology**

Two major physiological roles of melatonin are established in vertebrates. First, melatonin is involved in the circadian system. Second, melatonin is critical for the photoperiodic system to sense the environment and operate accordingly. Organisms use daily melatonin rhythm to decipher the photoperiodic message (LD cycle) of the environment.

**Melatonin in the circadian system**—A circadian 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. In mammals, the SCN of the anterior hypothalamus contains central circadian pacemaker and coordinates overt circadian rhythms\(^{14,15}\). In birds the circadian system is highly complex, as it comprises several independent clocks. Each clock has its own input and output pathways. Thus, birds perceive light by the retinae of the lateral eyes, by the pineal gland, and by the photoreceptors in the hypothalamus. Circadian oscillators are present at all the three levels, each having independent outputs. Unlike mammals, avian SCN is recognized in two sets of structures, medial (mSCN) and visual SCN (vSCN). Further, in birds these three central clocks interact with each other before producing the final output. Melatonin is a known output of at least two of them, the pineal gland and the eyes.

In the circadian clock system, melatonin performs at least two roles, although their relative importance may be species-specific. (1) Melatonin is a potential clock component. The retinal and pineal clocks control their circadian outputs via melatonin rhythm. Therefore, the absence of melatonin rhythm leads to arrhythmicity. (2) Melatonin acts as a coupling agent. It affects either the frequency of one or more oscillators or the strength of coupling among different oscillators, or both\(^{37-39}\). Any change that occurs in melatonin profile will, therefore, induce changes in the circadian system functions. A high-amplitude melatonin will strengthen the mutual coupling, resulting in an increase of the self-sustainment of clocks and hence decreases their susceptibility to the photoperiodic noise. The reduced melatonin amplitude will weaken the mutual coupling, resulting in a decrease of the self-sustainment of clocks and hence facilitating the adjustment of the circadian system to changing Zeitgeber conditions.

Most studies have measured the effects of pineal melatonin on circadian system regulated behavioural rhythms, especially locomotion and feeding activities. Surgical removal of the pineal (pinealectomy) disrupts circadian rhythmicity of locomotor activity\(^{40}\), body temperature\(^{41}\), and feeding\(^{38}\) in House Sparrows. However, the same effect is not achieved by neural disconnection of the pineal gland or by chemical sympathectomy\(^{42}\), which suggests that some humoral substance (melatonin) is involved. The pineal (melatonin) contains the circadian information since arrhythmic pinealectomized House Sparrow becomes rhythmic when the pineal gland from another bird is transplanted in its eye\(^{43}\). Periodically applied exogenous melatonin by infusion or by drinking water also restores rhythmicity in arrhythmic pinealectomized House Sparrows and Pigeons\(^{44-46}\), confirming that the clock properties of the pineal are achieved through the secretion of melatonin. The effect of pineal removal on circadian activity rhythms in the Indian Weaverbird (*Ploceus philippinus*) was studied. When Weaver birds were exposed to a 12L:12D Zeitgeber conditions, all of them showed good entrainment. Half of them were pinealectomized and the other half were sham-operated. Pineal removal led to the gradual loss of circadian rhythmicity in Indian Weaverbirds\(^{47}\), similar to that reported in House Sparrow.
The effect of pineal removal though is not uniform in the avian world. In European Starlings (Sturnus vulgaris), the role of pineal gland in the control of circadian behavioural rhythms is only partial. Pinealectomy impairs activity, but not feeding rhythms\(^\text{38}\). In Chicken (Gallus domesticus)\(^\text{59}\) and Japanese Quail\(^\text{70}\), the removal of pineal has no effect on circadian activity rhythms. Rather, removal of the eye (enucleation) impairs circadian rhythmicity in Quails. Since enucleation in Quail, achieved by sectioning the optic nerve, leaves rhythm in melatonin secretion intact\(^\text{51}\), the melatonin is not responsible for enucleation-induced arrhythmicity in Quails. In pigeons, pinealectomy and blinding together produces arrhythmicity, but none of them alone could achieve this. Cyclic melatonin infusions restored rhythmicity in arrhythmic individuals\(^\text{32}\).

The other evidence of melatonin being part of the avian circadian system comes from studies, which have manipulated the rhythm of melatonin. A change in the amplitude of melatonin, for example, will have consequential effects on the properties of avian circadian system, as one would predict from the general principles of an oscillator theory\(^\text{53}\). The persistence of circadian rhythms under constant conditions, the range of entrainment (i.e. the range of Zeitgeber periods to which rhythmicity can be synchronized), and the time required to re-synchronize following phase shifts of the Zeitgeber should change in absence of melatonin rhythm. Several recent studies clearly support this idea. In House Sparrows, the elimination of rhythm in plasma melatonin by pinealectomy which removed the elevated nighttime melatonin levels, or by melatonin implants which enhanced both the daytime and nighttime levels to supraphysiological levels, resulted in (i) loss of the circadian rhythmicity in locomotion and feeding\(^\text{38,54}\), (ii) an increase in the range of entrainment\(^\text{53,56}\), and (iii) a decrease in the duration required for resynchronization following phase shifts\(^\text{65,58}\).

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 kept under constant conditions and free-ran, but not when they were kept under LD and synchronized. Further, there was always a residual rhythmicity for-a-while in pinealectomized Sarrows\(^\text{40}\). On the other hand, lesions of the hypothalamic pacemaker resulted in severe impairment of rhythmicity in Sparrows although they had their pineals intact\(^\text{59}\). Collectively, this means that melatonin interacts with at least one oscillator other than its source to regulate avian circadian rhythmicity. In absence of melatonin signal, hypothalamic oscillator functions as a damped oscillator. In presence of light, however, hypothalamic oscillator continues to function as a self-sustained pacemaker even if the melatonin signal is absent.

**Pineal gland and clock genes expression**—Time is generated at molecular level within a clock structure by interlocking transcription—translation negative feedback loops, comprising positive and negative limbs. Each limb is formed by a set of core genes (called clock genes). In vertebrates, core clock genes of the positive limb are *bmal* (brain and muscle ARNT-like) and *clock* (circadian locomotor output cycles kaput), while that of negative limb are *periods* and *cryptochromes*. Pineal gland and melatonin have been shown to influence the expression of these clock genes in some but not in other species. In rat, pineal gland is not essential for circadian expression of *rper2* mRNA in SCN and peripheral tissues\(^\text{66}\) and in limbic forebrain system\(^\text{61}\). However, in PT of the rat *rcry1* expression is directly induced by melatonin\(^\text{62}\). House Sparrow vSCN contains melatonin receptor binding sites\(^\text{63}\). In Japanese Quail, constant exogenous melatonin administration caused arrhythmicity or period changes in body temperature and activity rhythms\(^\text{64}\), and rhythmic melatonin administration entrained feeding rhythm\(^\text{55}\), but melatonin did not alter the expression of clock genes in mSCN\(^\text{66}\). Also, pinealectomy failed to abolish the rhythmic expression of *period2* gene in House Sparrows\(^\text{67}\). Also, a differential effect of pinealectomy on rhythmic expression of *bmal1, period3* and *cryptochrome* in different brain areas was found in Chicken\(^\text{68}\). Because in these studies, gene expression was studied within three days of exposure to constant conditions, when birds were still behaviourally rhythmic\(^\text{10}\), the possibility remained that there was still residual melatonin effect on gene expression. It needs further investigation.

**Melatonin rhythm encodes photoperiodic information**—The duration of nocturnal melatonin production reflects night length, and hence day length\(^\text{73}\). On the other hand, the peak amplitude melatonin levels also appear to reflect the seasons. In House Sparrows, these levels are high in summer, low in winter and intermediate in spring\(^\text{29}\). Similarly, the...
amplitude of plasma melatonin rhythm is significantly reduced during migratory seasons in migratory Garden Warblers (Sylvia borin)\textsuperscript{69}, and during summer months in Adelie Penguin (Pygoscelis adeliae)\textsuperscript{70,71} and Arctic Svalbard Ptarmigan (Lagopus mutus hyperboreus)\textsuperscript{72}. A study on House Sparrows also suggested that the pineal gland could store and retain the photoperiodic information\textsuperscript{28}. Implication of all these findings is that melatonin rhythm provides the birds a calendar based on the photoperiodic information of the environment. The capacity of storing biologically meaningful information about time by the pineal may enable birds to compare and measure the day length, as season progresses, and to buffer the effects of adverse environmental conditions, when photoperiodic measurement may be tampered temporarily. This is supported by a recent study showing season-linked differences in the genes expression pattern at the transcript levels that encode enzymes of melatonin biosynthesis in Chicken pineal\textsuperscript{73}.

Melatonin and photoperiodic control of reproduction in birds—Because melatonin is a part of avian circadian system, it is assumed to be involved in the regulation of the photoperiodic effects at one or the other level. However, melatonin signal appears to be redundant in many bird species as far as photoperiodic effects on gonadal growth and development is concerned. The list of birds include European Starling (Sturnus vulgaris)\textsuperscript{74}, Spotted Munia (Lonchura punctulata)\textsuperscript{75}, American Tree Sparrow (Spizella arborea)\textsuperscript{76}, Japanese Quail (Coturnix c. japonica)\textsuperscript{77}, Blackheaded Bunting (Emberiza melanoccephala)\textsuperscript{77} and Redheaded Bunting (Emberiza bruniceps)\textsuperscript{78}. The absence of role of melatonin in photoperiodic induction of gonadal growth and development in birds could be explained by a study of Saldanha et al.\textsuperscript{79} on Ring Doves (Streptopelia roesogrisea). They showed direct innervation of GnRH neurons by deep brain photoreceptors (DBPs). This might mean that 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. Alternatively, these brain photoreceptors also contain clock, a speculation that needs to be investigated further.

The effects of pinealectomy or melatonin injections are shown nonetheless in some birds, including Domestic Duck (Anas platyrhynchos)\textsuperscript{80}, Indian Weaverbird (Ploceus philippinus)\textsuperscript{81}, Indian Jungle Bush Quail (Perdicula asiatica)\textsuperscript{82}, Rose-ringed Parakeets (Psittacula krameri)\textsuperscript{83} and Lal Munia(Estrilda amandava)\textsuperscript{84}. However, Kumar et al.\textsuperscript{78} reported absence of melatonin effects in certain phases of the gonadal growth and development cycle. The effect of melatonin as a modulator of testicular growth and development is also shown in Blackheaded Bunting. Buntings were implanted with melatonin-filled or empty silastic capsules subcutaneously and subjected to 11.75 h light per day (11.75L:12.25D) of long (red) or neutral (white) light wavelengths. Those exposed to long light wavelengths and melatonin-filled capsules had grown significantly larger testes\textsuperscript{85}. These results are consistent with the hypothesis that elimination of melatonin rhythm probably altered the perception of the day length. Trivedi et al.\textsuperscript{86} have also shown themodulatory effect of melatonin on photoperiodic induction of testicular growth in the Redheaded Buntings, in which exogenous injections of prolactin attenuated long day induction of the photoperiodic response\textsuperscript{87}.

However, in Japanese Quail, mediobasal hypothalamus (MBH) controls photoperiodic time measurement for reproductive function\textsuperscript{88}. As lesioning of MBH leads in the blocking of testicular recrudescence in response to increasing photoperiods and light illumination of this area resulted in testicular recrudescence\textsuperscript{90,91}. Rhythmic expression of the clock genes in the MBH led them to hypothesize that MBH containsa circadian pacemaker associated with the photoperiodic time measurement\textsuperscript{17,91}.

Melatonin and GnIH—In vertebrate system, gonadotropin secretion is under the control of hypothalamic peptide gonadotropin-releasing hormone (GnRH)\textsuperscript{92}. This gonadotropin secretion could be inhibited by another hypothalamic neuropeptide gonadotropin-inhibitory hormone (GnIH)\textsuperscript{93}. So far, these GnIH releasing neurons have been identified only in the PVN of birds\textsuperscript{93,96}. However, GnIH fibers are distributed throughout the hypothalamus and also in the median eminence (ME) suggesting GnIH to be involved in the regulation of anterior pituitary functions\textsuperscript{93,97}. Melatonin is shown to induce GnIH expression in the Quail brain\textsuperscript{98}. Decreased levels of GnIH precursor mRNA and peptide in pinealectomized and enucleated Quails have been to shown to be restored by melatonin administration in the dose-dependent manner\textsuperscript{98}. Dose-dependent effect of melatonin on GnIH release from
pineal gland. It is established that the photoperiodic information is relayed via rhythmic secretion of melatonin by the pineal gland, and is used in the regulation of reproductive cycle in mammals. Pinealectomized male Hamsters did not gonadally regress under non-stimulatory short photoperiods, and infusion of melatonin led gonadal regression in Siberian hamsters on stimulatory long days. Short day breeding sheep and goats also use melatonin in timing their reproduction, and melatonin can be used to advance or induce cyclicity in these seasonal breeders. The effect of melatonin is at the central level, i.e. at the level of hypothalamic-hypophysial axis. In anoestrous ewes, melatonin treatment over about 10 weeks induces a significantly high GnRH and LH pulsatility. Melatonin in the form of implants has been commonly used to alter the breeding season of short day seasonal breeders like ewes and goats. Melatonin alone or combined with photoperiod alters reproductive activity in anoestrous ewes. A study on rabbits showed that melatonin treatment improved the quantity and quality of ejaculate traits and overall sexual activity and decreased gestation period and pre-weaning mortality.

Melatonin and photoperiodic control of reproduction in mammals

After the discovery of melatonin, most early research was focused on its role in the regulation of reproduction in seasonal breeding rodents and sheep. It is established that the photoperiodic information is relayed via rhythmic secretion of melatonin by the pineal gland, and is used in the regulation of reproductive cycle in mammals. Pinealectomized male Hamsters did not gonadally regress under non-stimulatory short photoperiods, and infusion of melatonin led gonadal regression in Siberian hamsters on stimulatory long days. Short day breeding sheep and goats also use melatonin in timing their reproduction, and melatonin can be used to advance or induce cyclicity in these seasonal breeders. The effect of melatonin is at the central level, i.e. at the level of hypothalamic-hypophysial axis. In anoestrous ewes, melatonin treatment over about 10 weeks induces a significantly high GnRH and LH pulsatility. Melatonin in the form of implants has been commonly used to alter the breeding season of short day seasonal breeders like ewes and goats. Melatonin alone or combined with photoperiod alters reproductive activity in anoestrous ewes. A study on rabbits showed that melatonin treatment improved the quantity and quality of ejaculate traits and overall sexual activity and decreased gestation period and pre-weaning mortality.

Melatonin and secondary sexual characteristics

One well-studied secondary sexual character in which the role of melatonin has been studied is the development of song system in Passeriformes birds, commonly called as songbirds. The avian vocalization can be broadly characterized in two major categories—the song and call. A song is typically complex, long, multisyllabic and mostly produced by adult male birds, and is species-specific and can bear the signature of an individual. Usually short, monosyllabic call is also produced by male and female as well as juvenile birds for general communications such as in alarm, food pleading, food availability and other social communications. However, adult male birds generally use its song to attract a mating partner and protect their territory. In captivity, these male songbirds sing both undirected and directed song to attract the females, but in some situations as in case of predator—prey, such songs may not necessarily address to a conspecific. The avian vocalization is under control of complex circuit that includes a group of interconnected and well distinct nuclei, collectively called song control nuclei. The circuit comprises motor nuclei, involved in auditory feedback essential for vocal learning and perception. On the top of this pathway is a nucleus hyperstriatum ventrale, parscaudale (high vocal center, HVc) that leads to the nucleus robustus archistriatalis (RA). RA then leads to nucleus intercollicularis (ICo) and the Xth cranial nerve in the brainstem, which connects to the syrinx. RA and ICo also communicate to the nucleus retroambigualis (RAm) and nucleus ambiguous (NA), both help in the integration of song production. The HVc also communicates with RA through other pathway, which consists of a projection from HVc to Area X of the lobus parolfactorius (LPO) and medial portion of the dorsolateral nucleus of the anterior thalamus (DLM). DLM leads to lateral portion of the nucleus magnocellularis of the anterior neostriatum (IMAN) that finally connects to RA.

Seasonal variation in the size of nuclei of the song control system largely depends on the circulating testosterone level. Nonetheless, there is a testosterone independent seasonal variation in the size of song control nuclei. Melatonin is a key player in the steroid-independent neuromodulation of the song control nuclei induced by the photoperiod. The distribution of melatonin binding sites has been well identified in different regions of avian brain including the song control system. Whitfield-Rucker and Cassone showed 2-[125I]iodomelatonin (IMEL) binding in the HVc, RA and Area X in male, but not in female, House Sparrows. However, IMEL in the magnocellularis anterior (MAN) had melatonin binding sites in both sexes. There was also a significant difference in the IMEL binding under different photoperiods between HVc and RA. These IMEL binding studies clearly suggest the involvement of melatonin in the daily and seasonal regulation of
song in songbirds. This was confirmed through separate experiments by administering melatonin in birds kept under different photoperiods. Exogenous melatonin mitigates the long day induced increase in the volume of HVc and decrease in the volume of Area X, independent of the reproductive state, in European Starlings. House Sparrows maintained inconstant light (LL) and received long duration of melatonin cycle (14 h melatonin and 10 h no melatonin) exhibited smaller HVc and RA, but those on the same light treatment with a Shortmelatonin duration (8 h melatonin and 16 h no melatonin) exhibited smaller HVc and RA, but those effects were independent of the reproductive state of Sparrows.

The circadian clock can influence song learning as well as the timing of song production. There is growing evidence to suggest that circadian clock effect on bird song and learning behaviour are mediated through the rhythmic secretion of pineal melatonin. Wang et al., tested more directly this on Zebra Finches. They showed that pinealectomy led to the loss of circadian rhythmicity in the activity, song and call behaviour, and exogenous melatonin restored rhythmicity in all the three behaviours. Similarly, song in adult Zebra Finches and crowing in Japanese Quail was affected in adult birds when they were socially isolated for several weeks and transferred from a light-dark (LD) cycle to constant light (LL) condition, which caused loss of rhythmicity in the melatonin secretion. A recent study on roosters further confirms circadian clock control of crowing, but it is not known whether this is influenced by rhythmic melatonin secretion.

Pineal and non-reproductive seasonal behaviour

Many species of birds migrate to other continent to avoid hostile environment at home. Such night migratory birds when kept in captivity and hence disallowed migration exhibit migratory restlessness (phenotype by showing intense night time activity) or Zugunruhe, characterized by wing-whirring. Such migratory phenotype expression is reported to be under the endogenous clock control. Daily melatonin profile of migratory Garden Warbler showed corresponding changes in the simulated non-migratory and migratory conditions in the melatonin levels; night melatonin peak had a lower amplitude in migratory than in the non-migratory state. A similar higher amplitude of melatonin secretion in non-migratory than in the migratory was also reported in migratory Blackcaps (Sylvia atricapilla). Resident population of Blackcaps did not show seasonal changes in the amplitude of melatonin secretion. There is an effect of the absence of pineal on development of Zugunruhe in the migratory Blackheaded Bunting; pinealectomized birds show significantly delayed Zugunruhe on exposure to long days (unpublished data). However, a relatively recent study on migratory Garden Warbler showed that food availability rather than melatonin played a role in the development of nocturnal migratory restlessness.

Melatonin and human health

Melatonin has been implicated in various ways both indirectly and directly in human health. It is beyond the scope of this review to address in detail the role of melatonin in human health, but a few examples are given below to highlight the importance of melatonin in human physiology and diseases that appeared to be influenced by the biological clock.

Jet lag—Jet lag describes a temporary disturbance in the physiology of an individual due to loss of synchrony between internal circadian clock and external day-night condition, when the person travels across multiple time zones in short duration of time, e.g. flying to a country with different time zone. Most common symptoms that a jet lagged person will show are poor night's sleep, feeling groggy during the day and general unease and gastrointestinal stress. In general, travelling to the west has a lesser effect than travelling eastwards. This is probably because we catually delay our body clock by 2 h, but we can advance only by 1 to 1.5 h. Melatonin has been found to overcome jet lag effects. It is reported that 2-5 mg of melatonin prior to bedtime helps to overcome jet lag effects and improves sleep quality.

Sleep—Role of melatonin in sleep regulation has been widely studied, and the relationship between two appears complex. The widely accepted mechanism for melatonin effects on sleep is through the circadian system. Melatonin opens the sleep gate and helps to lower the body temperature; peak melatonin levels coincide with low body temperature later in the night. An interesting case study on the role of melatonin in sleep comes from a 14 year old boy with germ cell tumor that involved pineal region was found to have suppressed melatonin level sand severe insomnia. His sleep continuity was restored when he took melatonin at a dose of 2 mg in the evening for 2 weeks. In another case study of a 24 year old woman with
reduced and arrhythmic melatonin production, night sleep consolidation was achieved after eight weeks of treatment with 2 mg melatonin shortly before bedtime\(^\text{142}\). However, a survey on the effects of melatonin in sleep disturbances that included all age groups did not find significant and clinically relevant role of melatonin on sleep quality, efficiency and latency\(^\text{143}\). Nonetheless, latter meta-analysis (a meta-analysis is a method that reconstruct the results based on different published studies, and try to identify the patterns or other relationship that may come to light in the context of multiple studies) studies drew different conclusions. Results from 17 different studies with 284 subjects of older age group subjected to meta-analysis revealed that melatonin had increased sleep efficiency and reduced sleep onset time\(^\text{144}\). Similarly, another meta-analysis study concluded that melatonin treatment advanced endogenous melatonin and sleep-wake rhythms in subjects reported with delayed sleep phase disorder\(^\text{145}\). A recent meta-analysis demonstrated that melatonin decreased sleep onset latency, increased total sleep time and improved overall sleep quality in humans\(^\text{146}\). Overall, melatonin has been found effective in treating insomnia among elderly people\(^\text{147-150}\).

**Immune system**—The role of melatonin in influencing the activity of the immune system has been studied during last 15 years. In rodents, pinealectomy led to decrease\(^\text{151,152}\), and the administration of melatonin led to increase\(^\text{153}\) in weight of immune organs like thymus and spleen. Extirpation of pineal in newborn rats causes structural disorganization of thymus\(^\text{154}\). Pinealacotomy delayed the development of thymus, spleen and bursa and affected humoral immune response in Chicks\(^\text{155}\), and non-specific immunity in Chicken\(^\text{156}\) and Ring Doves\(^\text{157}\). In Japanese Quail, melatonin given in drinking water caused an increase in humoral immune responses without prior immunosuppression\(^\text{158}\). Pinealectomy of 4-5 weeks old C57BL/6 mice resulted in significantly reduced interleukin-2 (IL-2) production and NK cell activity\(^\text{159}\). Melatonin plays a role in the recovery of the immune circadian organization in arthritic rats\(^\text{160}\). Melatonin can act directly on immune system since immunocompetent have been shown having nuclear receptor for melatonin\(^\text{161}\), although non-receptor mediated actions of melatonin are also known.

**Cancer**—In the last two decades, the medical implication of melatonin has been studied in relation to treatment of tumors. Anti-cancerous properties of melatonin have been reported. It is suggested that melatonin may control tumor growth partially by acting as a natural anti-angiogenic molecule\(^\text{162}\). Most studies suggest melatonin action on tumor either by promoting the apoptosis of cancerous cells or by inhibiting the growth of cancerous cells. At a dose of 50 mg/kg body weight, melatonin reduced the viability and volume of Ehrlich ascites-carcinoma cells and increased the survival in female mice\(^\text{163}\). A low dose of IL-2 combined with melatonin prolonged survival time in untreatable advance hematologic malignancies on 12 human cancer patients\(^\text{164}\). Melatonin could also play a role of potential therapeutic drugs in specific lymphoproliferative diseases\(^\text{165}\). Melatonin reduces the viability of human myeloid HL-60 cells via induction of apoptosis through the regulation of Bax/Bcl-2 expression\(^\text{166}\). Melatonin promoted cell cycle arrest and apoptosis in cell lines representing different human lymphoid malignancies including Epstein-Barr virus (EBV)-negative BL, diffuse large B-cell lymphoma, follicular B non-Hodgkin's lymphoma and acute T-cell leukemia cells\(^\text{167}\). In combination with retinoic acid and somatostatin, melatonin enhanced anti-cancer activity and resulted in reduced number of cancerous cells through apoptosis activity in the MCF-7 breast cancer cells\(^\text{168}\).

**Conclusion and perspective**

Melatonin seems to have much of global effects on physiology of vertebrates, and has medical implications even for human health. Whereas melatonin research in years to come has potential of providing immense benefit to mankind, there is also great deal of importance of such research on non-human species, and in particular seasonal species like most, if not all, birds which appear to possess a highly complex and diverse, yet plastic, biological clock system. The properties of avian clock system, and also clock system in groups like reptiles, amphibians and fish, hitherto not widely investigated, may vary from species to species. Species-specific clock could have tremendous ecological implications, and may be a part of the survival strategy of birds. This may enable, for example, species sharing the same environment to have different temporal strategies in order to perform optimally. Melatonin appears to be a key component in this scheme in birds. In absence of melatonin, all bird clocks may become incapable of showing self-sustained circadian oscillations. We are
still far away, however, from understanding how melatonin exactly meets the demands of a multi-oscillatory avian circadian system to adjust to the temporal environment. Furthermore, the effect of melatonin on many aspects of bird behaviour (e.g. singing, nesting, parental care etc.) is yet to be investigated thoroughly. This may be essential to understand more comprehensively the effect of melatonin on birds life processes. Finally, most data on understanding more comprehensively the effect of melatonin on many aspects of bird behaviour (e.g. singing, nesting, parental care etc.) is yet to be investigated. The wild population remains to be investigated.

References

Robertson L M & Takahashi J S, Circadian clock in the cell culture: II. In vitro photic entrainment of melatonin oscillation from dissociated chick pineal cells, J Neurosci, 8 (1988b) 22.


Heilig M & Cassone V M, Circadian feeding and locomotor activity rhythms in European Starlings, Sturnus vulgaris, J Comp Physiol, 126 (1978) 123.


Underwood H, Barrett R K & Siopes T, Melatonin does link the eyes to the rest of the circadian system in quail: A neural pathway is involved, J Biol Rhythms, 5 (1990) 349.


Hau M & Gwinner E, Continuous melatonin administration accelerates resynchronization following phase shifts of a light-dark cycle, Physiol Behav, 58 (1995) 89.


70 Cockrem J F, Plasma melatonin in the Adelie penguin (Pygoscelis adeliae) under continuous daylight in Antarctica, J Pineal Res, 10 (1991) 2.


72 Reierth E, Van’t Hof TJ & Stokkan K A, Seasonal and daily variations in plasma melatonin in the high Arctic svalbard ptarmigan (Lagopus mutus hyperboreus, Gen Comp Endocrinol, 90 (1999) 119.


77 Kumar V, Effect of melatonin in blocking the response to a skeleton photoperiod in the black-headed bunting, Physiol Behav, 59 (1996b) 617.


82 Haldar C & Ghosh M, Annual pineal and testicular cycle in the Indian jungle bush quail, Perdicula asiatica, with reference to the effect of pinealectomy, Gen Comp Endocrinol, 77 (1990)150.


87 Tewary P D, Tripathi B K & Kumar V, Effects of exogenous prolactin on ovarian growth and fattening in the redheaded bunting, Emberiza bruniceps, Gen Comp Endocrinol, 52 (1983) 315.


100 McGuire N L, Kangas K & Bentley G E, Effects of melatonin on peripheral reproductive function: Regulation of testicular


atraicapilla), in Perspectives in comparative endocrinology: Unity and diversity, edited by HJToos et al. (Monduzzi, Bologna Italy) 2001, 295.


155 Rodriguez AB & Lea RW, Effect of pinealectomy upon the nonspecific immune response of the ring-dove (Streptopelia risoria), *J Pineal Res*, 16 (1994) 159.


