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Complex bird clocks

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The circadian pacemaking system of birds comprises three major components: (i) the pineal gland, which rhythmically synthesizes and secretes melatonin; (ii) a hypothalamic region, possibly equivalent to the mammalian suprachiasmatic nuclei; and (iii) the retinae of the eyes. These components jointly interact, stabilize and amplify each other to produce a highly self-sustained circadian output. Their relative contribution to overt rhythmicity appears to differ between species and the system may change its properties even within an individual depending, for example, on its state in the annual cycle or its photic environment. Changes in pacemaker properties are partly mediated by changes in certain features of the pineal melatonin rhythm. It is proposed that this variability is functionally important, for instance, for enabling high-Arctic birds to retain synchronized circadian rhythms during the low-amplitude *zeitgeber* conditions in midsummer or for allowing birds to adjust quickly their circadian system to changing environmental conditions during migratory seasons. The pineal melatonin rhythm, apart from being involved in generating the avian pacemaking oscillation, is also capable of retaining day length information after isolation from the animal. Hence, it appears to participate in photoperiodic after-effects. Our results suggest that complex circadian clocks have evolved to help birds cope with complex environments.

Keywords: behaviour; circadian rhythm; melatonin; pineal gland; suprachiasmatic nuclei

1. INTRODUCTION

Circadian rhythms, i.e. daily rhythms of endogenous origin, are fundamental for the temporal organization of the behaviour and physiology of all higher organisms (Saunders 1977; Aschoff 1981; Pittendrigh 1993). At the most basic level, such rhythms are generated within cells containing a specific 'molecular clockwork' (for a review, see Dunlap 1999) and forming functional units that drive rhythms in biochemical, physiological and behavioural functions. In mammals, the master clock controlling circadian rhythmicity is highly concentrated in the hypothalamic suprachiasmatic nuclei (SCN) (Klein *et al.* 1991; Silver *et al.* 1996; Moore & Silver 1998). Photic input is exclusively received from the retinae of the eyes and no other structure in the mammalian brain has yet been identified as containing a circadian oscillator (Menaker *et al.* 1997; Hastings & Maywood 2000).

In birds, the circadian pacemaking system appears to be more complex. It perceives information about the photic environment by retinal, pineal and deep encephalic photoreceptors (Cassone & Menaker 1984; Menaker *et al.* 1997; Foster & Soni 1998; Kojima & Fukada 1999), and it comprises at least three autonomous and anatomically distinct circadian clocks: the retina (Binkley *et al.* 1980; Underwood 1994); the pineal gland (Takahashi *et al.* 1980; Robertson & Takahashi 1988; Brandstätter *et al.* 2000); and a hypothalamic oscillator (Takahashi & Menaker 1979, 1982; Brandstätter *et al.* 2001*a*). To our present knowledge, all three components may contribute to regulate overt behavioural and physiological rhythmicity: (i) the pineal gland, by rhythmically releasing melatonin; (ii) the hypothalamic oscillator, possibly acting through neural output signals; and (iii) the retinae of the eyes, acting either via periodic melatonin secretion or neural output signals. Several lines of evidence suggest that these three components interact jointly with each other to produce a stable overt circadian rhythmicity (for reviews, see Cassone & Menaker 1984; Cassone 1990; Weaver 1999; Gwinner & Hau 2000). Besides birds, such a multi-oscillatory organization of the circadian pacemaking system can also be found in lower vertebrates (Menaker *et al.* 1997; Underwood 1989), indicating that the highly centralized system of mammals is an exception among vertebrates (Menaker *et al.* 1997).

In the following, we shall briefly summarize our current understanding of the avian circadian pacemaking system and test the central assumption that at least two components, the pineal gland and the putative hypothalamic oscillator, amplify and stabilize each other to produce a highly self-sustained circadian output. On the basis of these results, we then dwell on possible consequences of seasonal and latitudinal changes in the patterns of melatonin secretion for the functioning of the circadian pacemaking system as a whole. Finally, we use experimental results from isolated pineal glands to illustrate that the individual components of the avian circadian pacemaker are not only involved in generating rhythmicity but may contribute to the transduction of biologically meaningful information about time into complex circadian behaviour.

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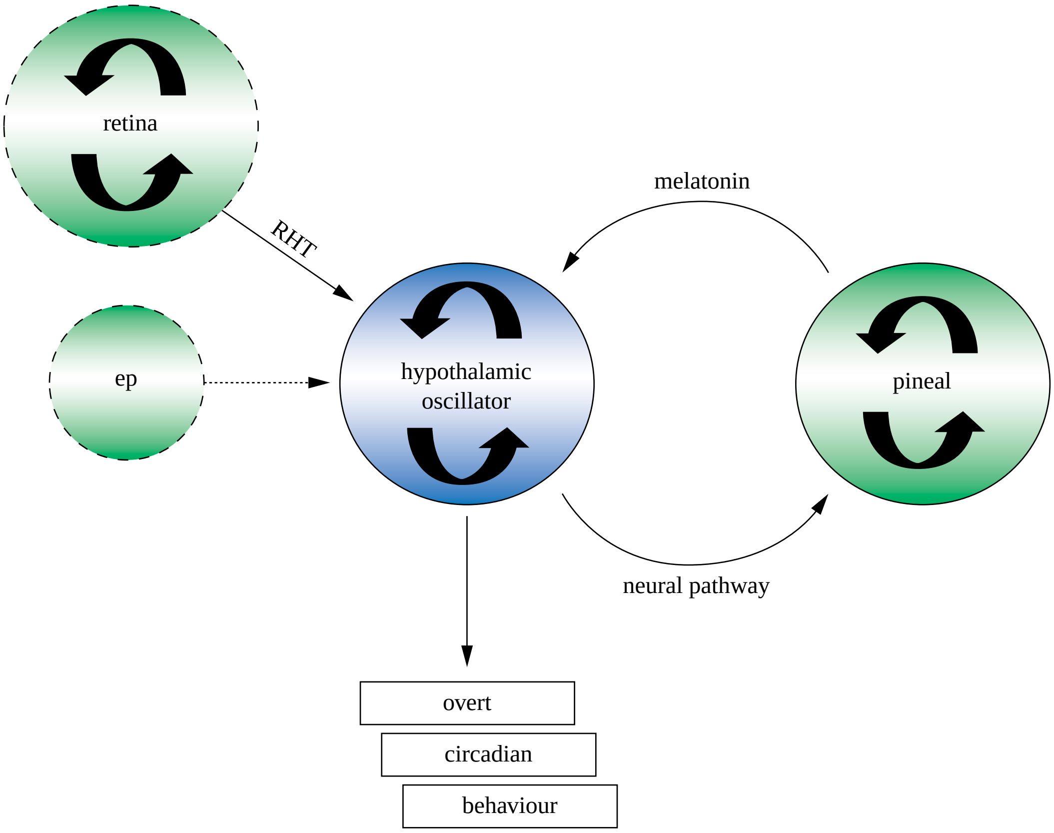

Figure 1. Diagram of the oscillatory components of the avian circadian pacemaker and some of their interactions, as well as sites of photoreceptors influencing the pacemaking system (green background). ep, encephalic photoreceptors; RHT, retinohypothalamic tract.

2. GENERAL PROPERTIES OF THE AVIAN CIRCADIAN PACEMAKING SYSTEM

Figure 1 shows a schematic diagram of the components of the avian circadian pacemaking system and possible ways in which they interact with each other. Central to the system are the pineal gland and a hypothalamic component possibly equivalent to the mammalian SCN (in the following called 'hypothalamic circadian oscillator'). The function and the significance of the pineal gland have been investigated in some detail. With one possible exception, the Japanese quail, all avian pineal glands so far studied contain an autonomous circadian oscillator that produces melatonin in a rhythmic fashion (Robertson & Takahashi 1988; Murakami *et al.* 1993; Taniguchi *et al.* 1993). This circadian rhythm of melatonin production, although different among species in the degree of its persistence, is maintained under constant conditions in cultured pineal glands (Takahashi *et al.* 1980; Brandstätter *et al.* 2000), dispersed pineal cells (Robertson & Takahashi 1988; Murakami *et al.* 1993) and even in isolated single pineal cells (Nakahara *et al.* 1997). In the house sparrow, the pineal oscillator is vital to the persistence of circadian rhythmicity *in vivo*. In birds kept under constant conditions, pinealectomy abolishes the circadian rhythms of locomotor activity (Gaston & Menaker 1968), feeding (Heigl & Gwinner 1994) and body temperature (Binkley *et al.* 1971). Rhythmicity can be restored when melatonin is rhythmically applied through injections (Gwinner & Benzinger 1978), infusions (Chabot & Menaker 1992) or the drinking water (Heigl & Gwinner 1994). It is also restored if the pineal gland of a sparrow is implanted into the anterior chamber of the eye of an arrythmic pinealectomized host. The emerging rhythm has the phase of the rhythm of the donor bird, indicating that circadian clock properties have been transplanted with the pineal gland (Zimmerman & Menaker 1979). However, following release of pinealectomized birds into constant conditions, house sparrow circadian rhythms usually do not disappear immediately but damp over a series of transient cycles, suggesting the existence of at least one other oscillator contributing to circadian pacemaking (Gaston & Menaker 1968). This conclusion is corroborated by the fact that in species other than the house sparrow pinealectomy renders circadian rhythmicity more sloppy but usually does not eliminate it (European starling, Janik *et al.* 1992), or leaves it entirely intact (quail, Simpson & Follett 1981; pigeon, Ebihara *et al.* 1987). The disruptive effects of electrolytic lesions of the anterior hypothalamus on circadian activity patterns in all species investigated until now (Takahashi & Menaker 1979, 1982; Simpson & Follett 1981; Ebihara & Kawamura 1981; Ebihara *et al.* 1987) and the recent demonstration of rhythmic clock gene expression in the house sparrow hypothalamus (Brandstätter *et al.* 2001) indicate that there is indeed a circadian oscillator in the hypothalamus of birds. The presence of melatonin-binding sites in this area suggests the possibility that the pineal melatonin rhythm might affect the hypothalamic oscillator (Cassone *et al.* 1995). Indeed, pinealectomy in house sparrows has resulted in a damping of the rhythm of hypothalamic 2-deoxyglucose uptake, concomitant with the fading away of behavioural rhythmicity (Lu & Cassone 1993), indicating that the pineal melatonin rhythm normally contributes to the persistence of the hypothalamic rhythm. However, a direct experimental demonstration that the hypothalamus contains an autonomous circadian oscillator acting as a pacemaker is still lacking. It must await the exact description of the complex functional anatomy of this region and *in vitro* work, as well as more elaborate lesioning and/or transplantation studies.

Just as the pineal rhythmicity affects the putative hypothalamic oscillator, the putative hypothalamic oscillator influences the rhythm in the pineal gland. Like the SCN in mammals it is connected to the pineal gland via a polysynaptic neural pathway (Ariens Kappers 1993). The interruption of this pathway, either by mechanical destruction of the superior cervical ganglion (Cassone & Menaker 1983) or by chemical sympathectomy with 6-hydroxydopamine (Zimmerman & Menaker 1975), disturbs or even abolishes the rhythm of melatonin production in the pineal gland. This suggests that the hypothalamic oscillator is involved in driving the rhythm in the pineal gland just as the pineal oscillator enhances the rhythm in the hypothalamus.

The third component of the circadian pacemaking system in birds, the retina, has so far been investigated in a few species only, particularly in the quail and in the pigeon. Like the avian pineal gland, the avian retina has been shown to produce melatonin rhythmically. However, in contrast to pineal melatonin, retinal melatonin is not released into the blood stream in all species (indicated, for example, by the fact that pinealectomy completely abolishes plasma melatonin in species like the sparrow and the starling). In species in which melatonin is secreted into the blood, it may (pigeon) or may not (quail) contribute to overt rhythmicity. In the pigeon, only the simultaneous elimination of the pineal gland and the eyes eliminates the plasma melatonin rhythm and, at the same time, results in behavioural arrhythmia (Ebihara *et al.* 1987). Behavioural rhythmicity can be restored by periodic application of exogenous melatonin (Chabot & Menaker 1992; Heigl & Gwinner 1994, 1995). In the quail, it also requires the simultaneous elimination of the pineal gland and the eyes to abolish both the plasma melatonin rhythm and the rhythms of behaviour. However, in this species, periodic melatonin secretion by the eye does not seem to be crucial because overt rhythms become abolished if only the optic tract is impaired, a treatment that leaves the plasma melatonin rhythm intact (Underwood *et al.* 1990; Underwood 1994).

Species differ not only with regard to the role of the eyes in the generation of pacemaker rhythmicity, but also with regard to the relative contribution of the pineal gland. In sparrows and some finches, the pineal gland appears to be the dominating component, as indicated by the fact that pinealectomy usually eliminates rhythmicity in constant conditions (Gaston & Menaker 1968; Beldhuis *et al.* 1988). In another passerine bird, the starling, pinealectomy results in the elimination of the locomotor activity rhythm in some individuals, whereas in the majority of birds it only destabilizes rhythmicity without eliminating it (Janik *et al.* 1992). Finally, in the quail and the pigeon, pinealectomy essentially has no effect at all (Simpson & Follett 1981; Ebihara *et al.* 1987). Likewise, the well-developed circadian system of the barn owl (*Tyto alba*) does not seem to depend on either the pineal gland or its periodic melatonin secretion because in this species the pineal organ is highly rudimentary (Quay 1972) and only traces of (arrhythmic) melatonin are found in the blood (Van't Hof *et al.* 1998).

Table 1. Summary of experiments testing predictions of the model of 'interval resonance'.

treatment	effect	reference
performance in constant conditions		
melatonin implant	arrhythmic	Turek *et al.* (1976)
pinealectomy	arrhythmic	Gaston & Menaker (1968); Gwinner (1989); Weaver (1999)
range of entrainment		
melatonin implant		
light as *zeitgeber*	larger	Hau & Gwinner (1994)
food as *zeitgeber*	?[a]	Gwinner *et al.* (1994)
pinealectomy		
light as *zeitgeber*	larger	E. Gwinner (unpublished data)
food as *zeitgeber*	larger	Heigl & Gwinner (1999)
resynchronization time		
melatonin implant		
light as *zeitgeber*	shorter	Hau & Gwinner (1995)
food as *zeitgeber*	shorter	Abraham *et al.* (2000)
pinealectomy		
light as *zeitgeber*	(shorter)[b]	V. Kumar and E. Gwinner (unpublished data)

[a] Small data set.

[b] Significant only for phase delay.

In summary, some common properties are detectable in the circadian pacemaking system of birds (figure 1), such as the presence of a multiple photic input (from three distinct sources) and a certain functional compartmentalization (exemplified by the presence of at least three autonomous circadian oscillators). Conversely, there is also a high degree of diversity, particularly in the role the individual components have in producing highly self-sustained circadian rhythms of behaviour. This remarkable interspecific diversity of the circadian pacemaking system in birds still awaits functional, and possibly ecological, explanations.

3. THE MODEL OF 'INTERNAL RESONANCE' AND PREDICTIONS ARISING FROM IT

The information available on the circadian pacemaking system of passerine birds suggests that at least the hypothalamic circadian oscillator and the pineal gland exert mainly enhancing effects on each other. It has therefore been suggested that these two oscillators jointly stabilize and amplify each other by resonance to produce a high-amplitude, highly self-sustained circadian output (Gwinner 1989). This implies that the elimination or reduction of the amplitude of one of the components of the system results in the reduction of the amplitude of the

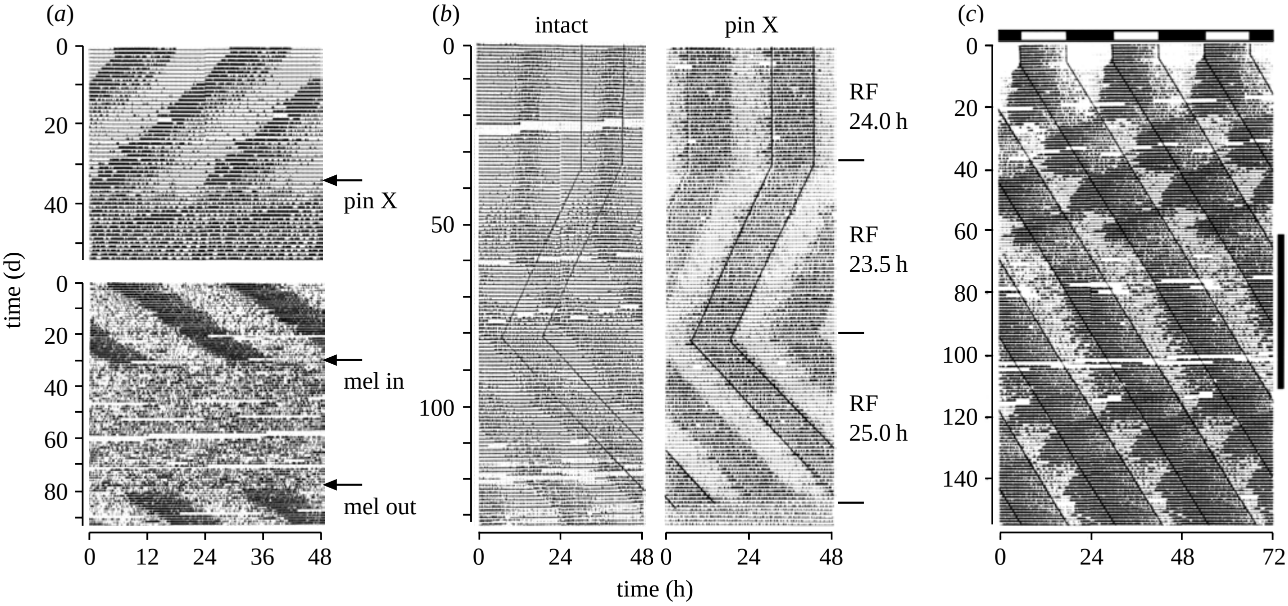

Figure 2. Effects of pinealectomy and/or melatonin treatment on circadian activity rhythms of house sparrows. (*a*) Locomotor activity rhythms of two house sparrows in constant darkness. The bird used in the upper diagram was pinealectomized ('pin X') at the time indicated by the arrow. The bird used in the lower diagram carried a silastic implant filled with melatonin between the days indicated by 'mel in' and 'mel out'. Both pinealectomy and melatonin implantation rendered activity arrhythmic (upper diagram from Gwinner *et al.* (1997); lower diagram from Turek *et al.* (1976)). (*b*) Circadian activity rhythm of an intact (left) and a pinealectomized (pin X; right) house sparrow kept in dim light (0.3 lux) and exposed to restricted feeding schedules (RF) with periods of 24.0, 23.5 and 25.0 h. During the initial 24.0 h cycle, food was available each day from hours 6 to 18. When the *zeitgeber* period was changed to 23.5 h and 25.0 h, respectively, the daily onsets and ends of feeding times were advanced by 1.5 h or delayed by 1.0 h, as indicated by the diagonal lines. During exposure to a *zeitgeber* period of 24.0 h and 25.0 h, the rhythms of both birds were synchronized. During exposure to the *zeitgeber* period of 23.5 h, only the rhythm of the pinealectomized bird was synchronized, whereas that of the intact bird was free-running, suggesting a wider range of entrainment in the pinealectomized than in the intact bird (after Heigl & Gwinner 1999). (*c*) Locomotor activity rhythm of a house sparrow kept under a low-amplitude light–dark cycle (2:1 lux) with a period of 24.5 h. The actogram is triple-plotted. Bars above each actogram indicate light–dark cycles. Diagonals in the actogram connect onsets and ends of light. During the interval indicated by the black bar at the side of the actogram, the bird carried a silastic capsule filled with melatonin. The bird initially showed 'relative coordination'. The implantation of melatonin led to full synchronization (after Hau & Gwinner 1994).

other component(s). According to the model, an experimental elimination of the endogenous melatonin rhythm should reduce the degree of self-sustainment of the hypothalamic circadian oscillator and, consequently, cause damping or loss of overt behavioural rhythms that are assumed to be directly controlled by it. According to general oscillator theory (Wever 1962, 1965), this should have the following consequences: (i) under constant conditions, rhythmicity should tend to be damped or, at least, lose its stability; (ii) in the synchronized state, (a) the range of entrainment, i.e. the range of *zeitgeber* periods to which rhythmicity can be synchronized, should increase, and (b) resynchronization times following phase shifts of a *zeitgeber* should decrease, i.e. rhythmicity should adjust faster to the new phase of the *zeitgeber*.

These predictions have been experimentally tested in house sparrows in which the melatonin rhythm was abolished, either by surgically removing the pineal gland, a procedure that eliminates the rhythm of circulating melatonin (Janik *et al.* 1992) or by 'masking' the endogenous rhythm of circulating melatonin with constant high levels of exogenous melatonin applied through silastic implants. Melatonin implants raise melatonin levels in the blood by a factor of 10–100 above normal average levels and, at the same time, eliminate the endogenous melatonin rhythm (Beldhuis *et al.* 1988; Abraham *et al.* 2000). In experiments testing for the effects of these treatments on circadian performance in the synchronized state, birds were usually synchronized with light–dark cycles. However, in some cases, a food *zeitgeber* rather than a light *zeitgeber* was used to avoid problems of interpretation resulting from the fact that the avian pineal gland, apart from being the seat of an oscillator, also contains photoreceptors.

The results of these experiments generally support the predictions (table 1): in the free-running state under constant conditions, both pinealectomy and application of melatonin through silastic tubing rendered activity of house sparrows arrhythmic (figure 2*a*). In synchronized conditions, the range of entrainment increased following pinealectomy, irrespective of whether the birds were exposed to a light or a food *zeitgeber* (figure 2*b*). Melatonin implantations clearly increased the range of entrainment to a light *zeitgeber* (figure 2*c*). Some preliminary data suggest that this may not be true for a food *zeitgeber*; however, only a very few birds have yet been studied under such conditions and more data are required to clarify the situation. Resynchronization times following 8 h advance or delay phase shifts were clearly shorter in melatonin-implanted birds than in controls, irrespective

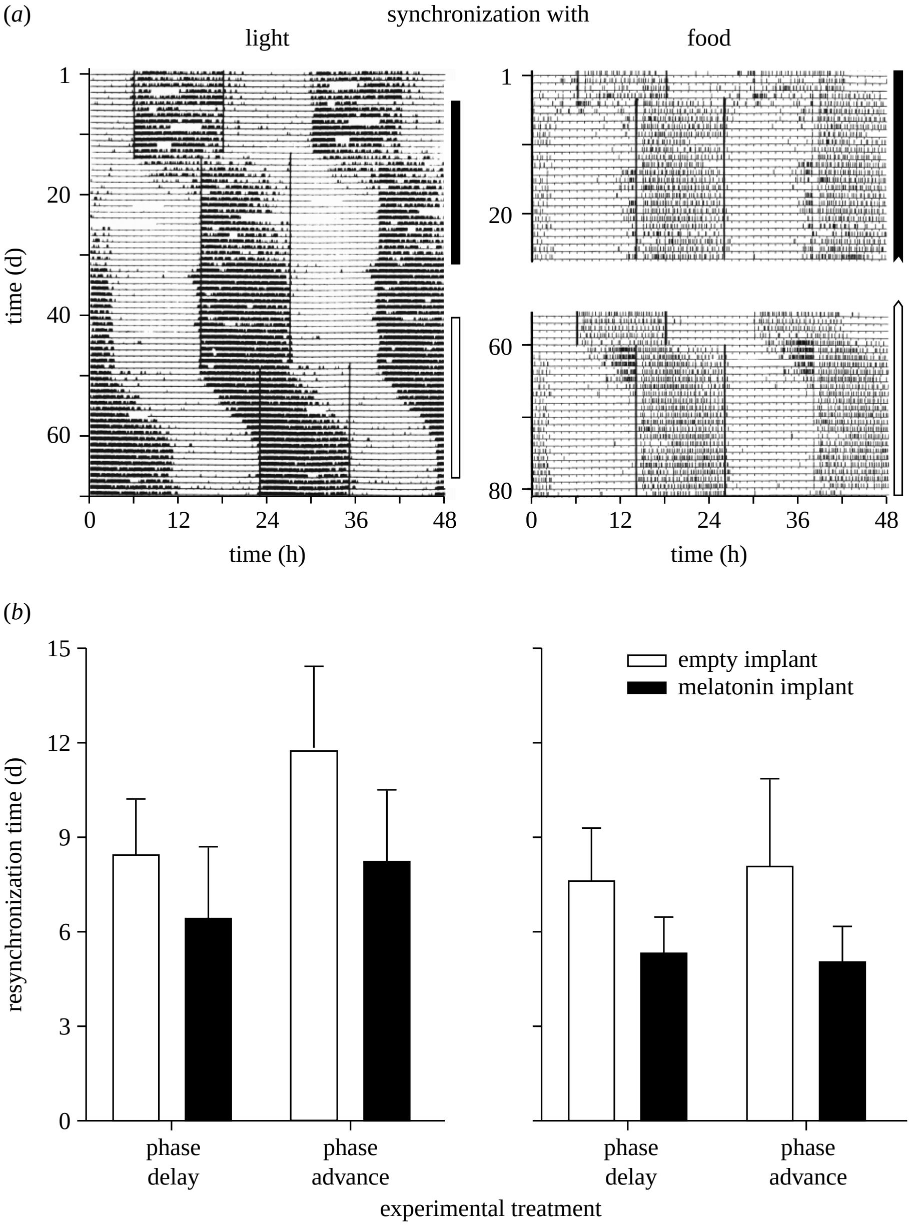

Figure 3. (*a*) Double-plotted actograms of the activity rhythms of two house sparrows kept either in a 12 L:12 D cycle (4:1 lux; left) or in a 12:12 h cycle of food availability and unavailability (right). At the times indicated, the *zeitgeber* cycles were phase-delayed by 8 h. During the periods marked by black vertical bars at the right-hand side of the actograms, the birds carried a melatonin implant; during the periods marked by the open bars, they carried an empty implant. (*b*) Mean resynchronization times (mean ± s.d.) of all birds in the two experiments after delay (left) or advance (right) phase shifts while carrying empty (white columns) or melatonin-filled (black columns) silastic capsules. When birds carried a melatonin implant, resynchronization times were significantly shorter than when carrying an empty implant (after Hau & Gwinner (1995) and Abraham *et al.* (2000)).

of whether a light or a food *zeitgeber* was used (figure 3). Experiments testing for the effects of pinealectomy on resynchronization time are still incomplete but preliminary data suggest that pinealectomized sparrows take less time to become resynchronized to a phase-shifted light *zeitgeber* than pineal-intact birds, at least following phase delays.

4. POSSIBLE RELEVANCE FOR UNDERSTANDING MELATONIN PATTERNS IN FREE-LIVING BIRDS

The results described in the previous section may assist the interpretation of variations in the daily melatonin pattern of animals living under natural or semi-natural conditions. Drastic reductions in the amplitude of plasma melatonin rhythms have been described for three situations.

(a) *Midsummer in high Arctic birds*

The amplitude of the 24 h melatonin rhythm was drastically reduced around midsummer in penguins (*Aptenodytes forsteri*; *Pygoscelis adeliae*) from Antarctica and Svalbard ptarmigans (*Lagopus mutus hyperborealis*) from the Arctic region (Miché *et al.* 1991; Cockrem 1991; Reierth *et al.* 1999). An example is shown in figure 4*a*. During the Arctic midsummer, irradiance is high and varies only slightly in the course of the day, and it is likely that the high irradiances suppress melatonin secretion. According

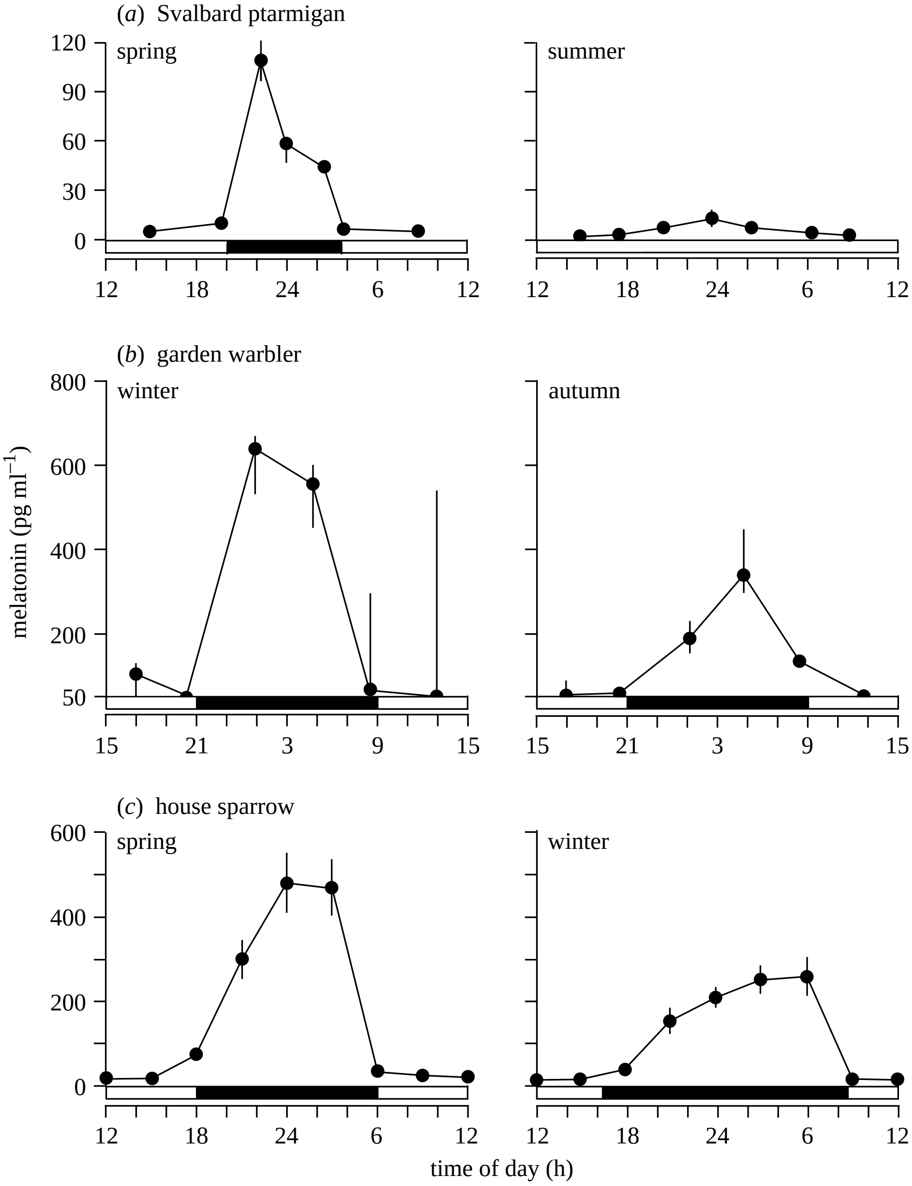

Figure 4. Examples of seasonal differences in the 24 h pattern of plasma melatonin. (*a*) Svalbard ptarmigan (*Lagopus mutus hyperborealis*) studied near Tromsø (70° N) under outdoor conditions in spring and summer (after Reierth *et al.* 1999). (*b*) Garden warbler (*Sylvia borin*) kept in a constant 12h photoperiod in autumn, when birds exhibit intense nocturnal migratory activity and during the quiescent period in winter when birds show no migratory activity (after Gwinner *et al.* 1993). (*c*) House sparrow (*Passer domesticus*) kept outdoors near Andechs (48° N) in spring and in winter (after Brandstätter *et al.* 2001*a*). Symbols in (*a*) and (*c*) indicate means ± s.e.m., and in (*b*) indicate medians with quartiles. Black bars, night.

to the results and considerations presented in the previous section, this, in turn, might reduce the degree of self-sustainment of the circadian pacemaker, thereby increasing its range of entrainment. As shown in figure 5, an increased range of entrainment should facilitate synchronization to weak, low-amplitude *zeitgebers*, such as prevail in the Arctic summer. Such a mechanism might explain the fact that free-running circadian rhythms have hardly (if ever) been observed among Arctic or sub-Arctic birds. Instead, most birds either exhibit 24 h rhythms or are arrhythmic (Remmert 1965).

(b) *Migratory seasons in migratory birds*

Studies on captive garden warblers (*Sylvia borin*) (Gwinner *et al.* 1993) and stonechats (*Saxicola torquata*) (E. Gwinner and T. J. Van't Hof, unpublished data) have revealed that the amplitude of the plasma melatonin rhythm becomes reduced by about 30% during the migratory seasons, when these birds exhibit nocturnal restlessness, as compared with other times of the year. These amplitude changes occur spontaneously and in parallel with the annual waxing and waning of migratory restlessness even in birds kept in a constant 12 h photoperiod (figure 4). However, they also depend on environmental conditions: in migratory garden warblers, the melatonin amplitude characteristic of the non-migratory season can be restored by exposing birds to a feeding schedule that interrupts migratory restlessness (Gwinner 1996). According to the model described above, the reduced amplitude of the melatonin rhythm should indicate a reduced degree of self-sustainment of the pacemaker, which should facilitate adjustment to changing *zeitgeber* conditions. Such a mechanism might be highly adaptive for migratory birds, which actively change environmental *zeitgeber* conditions as a result of their own migratory flights. The advantage of this mechanism should be particularly pronounced in birds that migrate fast and along an east–west axis. Preliminary results of experiments with garden warblers and bramblings suggest that resynchronization times are in fact shorter during than before and after the migratory seasons (Pohl 2000; E. Gwinner, unpublished data).

(c) *Winter in non-migratory birds*

House sparrows were investigated during the time of increasing photoperiod in spring, during the longest day length in early summer, around the equinox in autumn and during the shortest photoperiod in winter (Brandstätter *et al.* 2001*b*). There was a significant difference in peak amplitude levels of circulating melatonin. Amplitudes were highest and similar in spring and summer, intermediate in autumn, and lowest in winter (figure 4*c*). The reduced melatonin amplitude found in house sparrows in winter might indicate that the system is more passive at that time of the year and, thus, can more readily respond to increasing day lengths in late winter and early spring. The high-amplitude melatonin rhythm in spring and summer might reflect a more strongly self-sustaining circadian pacemaker. This may be important for stabilizing the circadian system and thereby reducing the effects of photoperiodic noise that might impair precise photoperiodic time measurement during this crucial time of the year.

5. CONSERVATION OF MELATONIN SECRETION PATTERN *IN VITRO*

The pineal melatonin rhythm is not only one of the components generating the avian pacemaking oscillation—it also appears to be involved in more sophisticated ways in regulating complex circadian performance. This is suggested by recent results indicating that day length information acquired by house sparrows and reflected in the pattern of melatonin release *in vivo* is retained for some time in isolated pineal glands cultured *in vitro* (Brandstätter *et al.* 2000). As in mammals, the duration of elevated melatonin in the plasma of sparrows is longer in birds kept under short day (=long night) conditions than in birds kept under long day (=short night) conditions (figure 6). In addition, the amplitude of the rhythm is low in the long night and high in the short night conditions. These different patterns persist for at least two cycles in birds released from the two photoperiods into constant darkness (figure 6); when pineal glands were explanted from sparrows kept in these conditions and cultured *in vitro*, the pattern of melatonin release continued to show these different patterns for one or two cycles.

These data indicate that one of the important components of the avian circadian pacemaking system, the pineal gland, can store and retain biologically meaningful information about time. Similar capacities have recently also been demonstrated for the mammalian SCN (Mrugala *et al.* 2000). The temporarily stored information might be used by birds to determine whether day length increases or decreases. It could also allow birds to buffer the effects of weather-dependent short-term variations in photoperiod, which might impair precise photoperiodic time measurement. Moreover, the capacity of the pineal gland to retain day-length-related information may be one of the mechanisms underlying photoperiodic after-effects on activity patterns, i.e. the fact that day-active birds that were initially exposed to long photoperiods retain longer activity times when transferred to constant light or darkness than animals previously exposed to short photoperiods (Wolfson 1966; Gwinner 1980). Such photoperiodic after-effects on behaviour, however, last longer than those on the patterns of melatonin, indicating that the former can be only partly explained by the latter. Nevertheless, the results provide initial evidence that circadian memory phenomena may, to some extent, be based on processes that can be investigated at the level of individual components of the circadian pacemaker and, hence, open the door for exciting future experimental work.

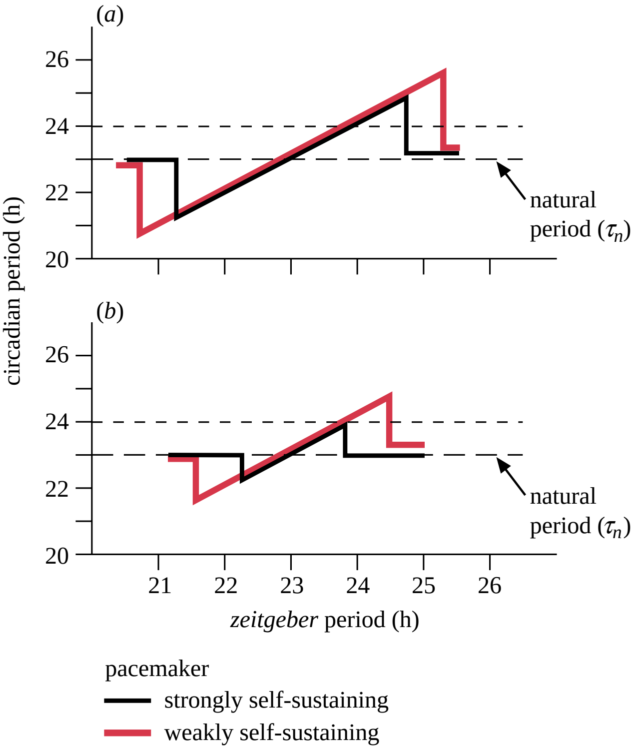

Figure 5. The range of entrainment of a strongly self-sustaining (black) and a weakly self-sustaining (red) pacemaker exposed to either (*a*) a strong or (*b*) a weak *zeitgeber*. The ordinate shows the period assumed by the pacemaker under the various *zeitgeber* periods plotted on the abscissa. During synchronization (i.e. within the range of entrainment), the period of the pacemaker is identical with the period of the *zeitgeber*. Beyond the limits of the range of entrainment, the rhythm free-runs with its characteristic natural period τ_n. During the equinoxes when the light–dark ratio is close to 1, *zeitgeber* conditions can be assumed to be stronger (*a*) than during midsummer when the light–dark ratio is much larger than 1. The range of entrainment is wider under the strong than under the weak *zeitgeber* (*a* versus *b*) and wider for the weakly self-sustaining (red) than for the strongly self-sustaining (black) pacemaker (partly after Wever 1962).

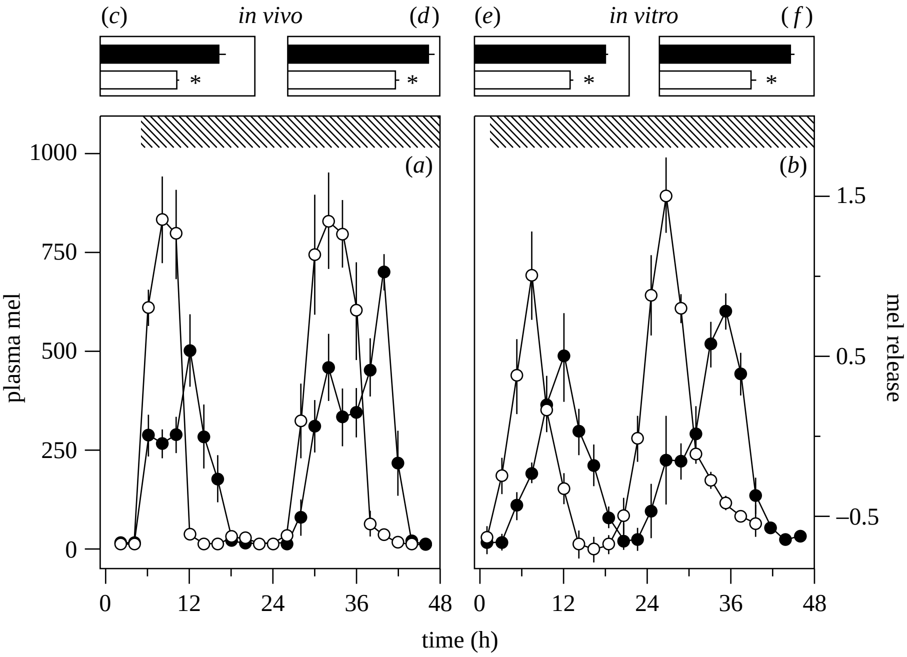

Figure 6. *In vivo* and *in vitro* melatonin production in the house sparrow during constant conditions after previous synchronization to different photoperiods. (*a*) *In vivo* melatonin profiles during 48 h in constant conditions following synchronization with two distinct photoperiods. Symbols represent mean values ± s.e.m. of melatonin (mel) in pg ml^{-1} plasma. (*b*) Profiles of relative melatonin release from isolated pineal glands of the two photoperiodic groups ($n = 8$ per group) during the first two cycles of culture in continuous darkness. Durations of elevated melatonin as well as amplitude values reflect the previous *in vivo* situation. Open circles, birds pretreated with 16 L:8 D; black dots, birds pretreated with 8 L:16 D. Hatched bars indicate constant darkness. The horizontal bars at the top represent mean values ± s.e.m. of the durations of elevated melatonin during the first (*c*) and second (*d*) cycle *in vivo*, as well as during the first (*e*) and second (*f*) cycle *in vitro*. The asterisks represent $p < 0.001$ (modified from Brandstätter *et al.* 2000).

6. CONCLUSIONS

Compared with mammals, birds are equipped with a rather complex and diverse circadian pacemaking system. It varies greatly among species and it appears to be flexible within a species and even within an individual bird. The latter is particularly obvious for the pineal melatonin component of the circadian pacemaker, which, depending on season or latitude, is either characterized by a high- or a low-amplitude rhythm of melatonin secretion. The conclusion that these changes result in corresponding changes of the amplitude of the pacemaker as a whole is supported by a series of experimental results obtained in laboratory experiments with house sparrows. The more far-reaching suggestion, that the variations in melatonin amplitude observed in free-living birds also reflect changes in overall pacemaker properties, is tempting but still awaits rigorous experimental testing. It therefore remains to be seen whether the reduced amplitude of the plasma melatonin rhythm found in birds during the migratory seasons or during the Arctic summer is, in fact, associated with an improved entrainability. The general proposition that the avian circadian pacemaker can indeed change its properties within an individual, for instance seasonally, is based on more solid grounds (e.g. Pohl 1994). Our present data suggest that changes of this kind may have adaptive value. In other words, the complexity of bird clocks may be related to the complexity and diversity of the various lifestyles birds have evolved in a variety of different environments.

At another level, we have presented evidence that the well-known capacity of circadian systems to temporarily store environmental information is partly a property of the pineal gland: photoperiodic information reflected in the daily duration of elevated melatonin is retained for at least two cycles in isolated pineal glands cultured *in vitro* under constant conditions. The capacity to 'memorize' daily environmental patterns may provide the basis for phenomena like photoperiodic after-effects on locomotor activity, carry-over phenomena in photoperiodic time measurement or even highly sophisticated performances like circadian time–place learning (Brandstätter *et al.* 2000). Our results are encouraging in that they suggest that it may be possible to unravel the physiological basis of such circadian memory phenomena. A possible first step, exemplified by our study on the avian pineal gland (Brandstätter *et al.* 2000), as well as investigations of the mammalian SCN (Mrugala *et al.* 2000), consists of studying the question of whether these phenomena are partly localized in known components of the circadian system.

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