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# Synchronization of a Circadian Rhythm in Pinealectomized European Starlings by Daily Injections of Melatonin*

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**Summary.** We have tested the hypothesis that the avian pineal hormone, melatonin, which is produced in and secreted from the pineal body in a circadian temporal pattern is a chemical mediator which drives overt circadian functions. Pinealectomized European starlings (*Sturnus vulgaris*) kept in continuous dim light, received intramuscular injections of melatonin at the same time each day for several weeks. Control birds received only sesame oil injections. In 21 out of 22 birds, melatonin treatment resulted in the synchronization of locomotor activity with the 24 h injection rhythm. In contrast, activity of only one out of 10 control birds became synchronized with the daily control injections. These results are consistent with the hypothesis that the endogenous circadian rhythm of melatonin concentration provides an internal synchronizing agent, acting directly on other circadian oscillators. Alternatively, it seems possible that the exogenous rhythm exerts its effect indirectly by modifying the sleep-wake cycle.

## 1. Introduction

The pineal gland plays a central role in the control of circadian rhythms in at least some species of birds. In house sparrows kept in continuous darkness (DD), the circadian rhythms of locomotor activity and body temperature are abolished when the pineal is surgically removed (Gaston and Menaker, 1968; Binkley et al., 1971). Reimplantation of a pineal into the anterior chamber of the eye restores the locomotor activity rhythm (Zimmerman and Menaker, 1975); the induced rhythm emerges with the phase of the rhythm of the donor (Zimmerman, 1976). These results provide strong evidence that the pineal body contains a circadian pacemaker. Recent findings in the European starling (*Sturnus vulgaris*) differ from those obtained in the house sparrow in so far as pinealectomy in that species usually leads only to heavy disturbances of the circadian activity rhythm rather than to its complete abolition (Gwinner, 1977, 1978; Rutledge and Angle, 1977). Nevertheless, they are consistent with the hypothesis that the pineal organ is the seat of a circadian pacemaker.

If the pineal gland is a circadian pacemaker, it most probably transmits its circadian information to the subordinate systems humorally. This is suggested by the findings in the house sparrow (1) that interruption of the only known neuronal output through the pineal stalk has no effects on the circadian activity rhythm; and (2) that re-implantation of a pineal into a pinealectomized arrythmic sparrow results in an immediate restoration of circadian rhythmicity (Zimmerman and Menaker, 1975). An obvious candidate for a substance conveying circadian information from the pineal gland to other components of the circadian system is melatonin (e.g. Menaker and Zimmerman, 1976). This indoleamine is synthesized in the pineal body and its concentration there as well as in the serum shows a pronounced circadian rhythm. In chickens, plasma and brain concentrations of melatonin are low during the active phase of the daily cycle of locomotor activity and high during the inactive phase (Pang et al., 1974; Pelham, 1975; Pelham and Ralph, 1972; Ralph, 1976; Ralph et al., 1974). – Recently it was shown that continuous administration of melatonin changes at least two parameters of the freerunning circadian activity rhythm in house sparrows, suggesting that melatonin is, indeed, involved in the physiological control of circadian rhythms (Turek et al., 1976). – To test the hypothesis that melatonin is a substance by which the pineal body drives overt circadian rhythms we have attempted to syn-

* Dedicated to Professor Colin S. Pittendrigh on the occasion of his 60th birthday

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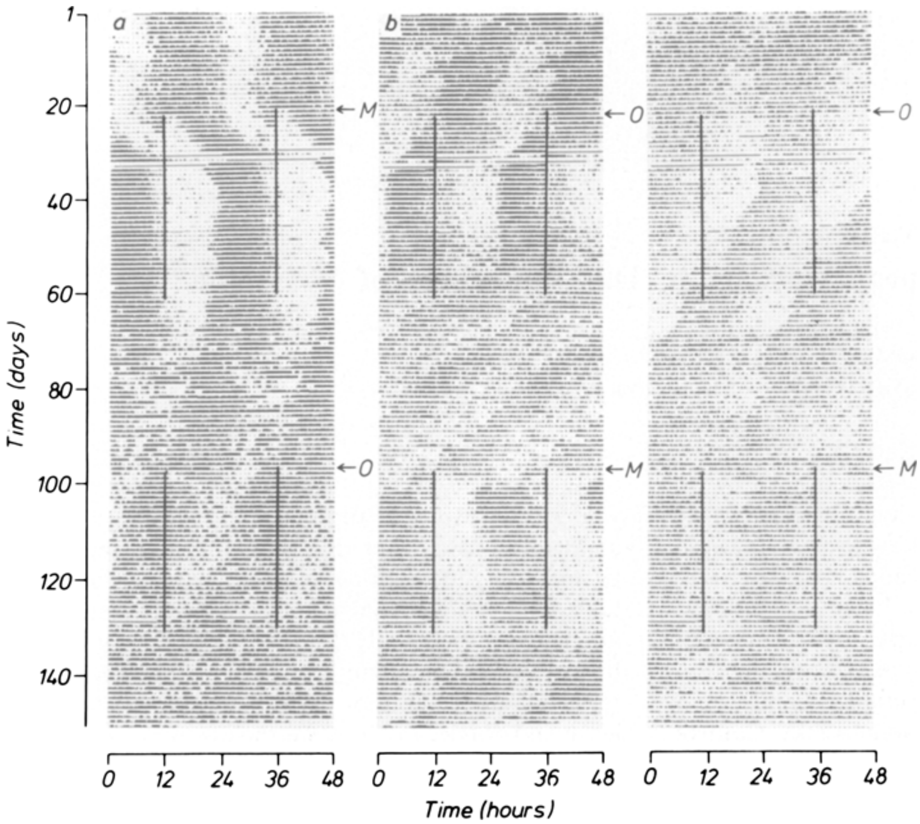


**Fig. 1.** Activity recordings of 3 pinealectomized starlings maintained in continuous dim light. Each horizontal line from hour 0 to 24 represents activity record of one day. Records of successive days are mounted underneath each other. To facilitate inspection of the data the records have been double-plotted on a 48-h time scale. Vertical marks indicate activity within 1 min time intervals. During times of intense activity marks fuse into a black block. The vertical lines connect the times of day at which the birds were injected with melatonin (*M*) or sesame oil (*O*)

chronize locomotor activity in pinealectomized European starlings with daily injections of this hormone.

## 2. Methods

24 starlings were pinealectomized as described elsewhere (Gwinner, 1978) and subsequently transferred to constant condition chambers. They were kept, 1 to 5 birds per chamber, in individual activity recording cages in which their locomotor activity was continuously recorded by microswitches mounted under one of the two perches. The impulses were stored on magnetic tape and later processed by a computer (for details see Daan, 1976).

Following transfer to constant condition chambers, the birds were first left undisturbed for at least 30 days either in constant darkness (DD) (9 birds) or in continuous dim light of about 0.01 lux (15 birds). Subsequently, they were injected intramuscularly with either 50 μg of melatonin dissolved in 0.1 ml of sesame oil or with 0.1 ml sesame oil only, for 27 to 38 successive days, at the same time each day. They were then left undisturbed for about 35 days. 22 birds were treated with melatonin and 10 with oil. After the indisturbed period of about 35 days, 8 of the birds were again treated with either melatonin or oil. 5 of these birds had first been treated with melatonin and then received oil; the other 3 birds had first been injected with oil and then with melatonin.

At the end of the experiment the birds were killed and their brains fixed for subsequent histological examination (Gwinner, 1978, for details). In most cases no pineal remnants could be detected. Only in a few brains were there parenchymal proliferations for which a pineal origin could not be excluded with certainty. However, because the behavior of these birds was indistinguishable from that of the others they were not excluded from the analysis.

## 3. Results

As in a previous study (Gwinner, 1978) most pinealectomized starlings in this experiment retained a clear, although in many cases rather "sloppy" circadian rhythm of locomotor activity. Only the activity of 3 birds was apparently arrhythmic for some time.

Among the 10 birds treated with oil alone, only one became synchronized with the 24-h injection rhythm (Fig. 1b). The rhythms of 2 birds showed signs of relative coordination (Fig. 1c). The activity patterns of the other 7 birds were not significantly affected by the oil treatment (Fig. 1a). In contrast,

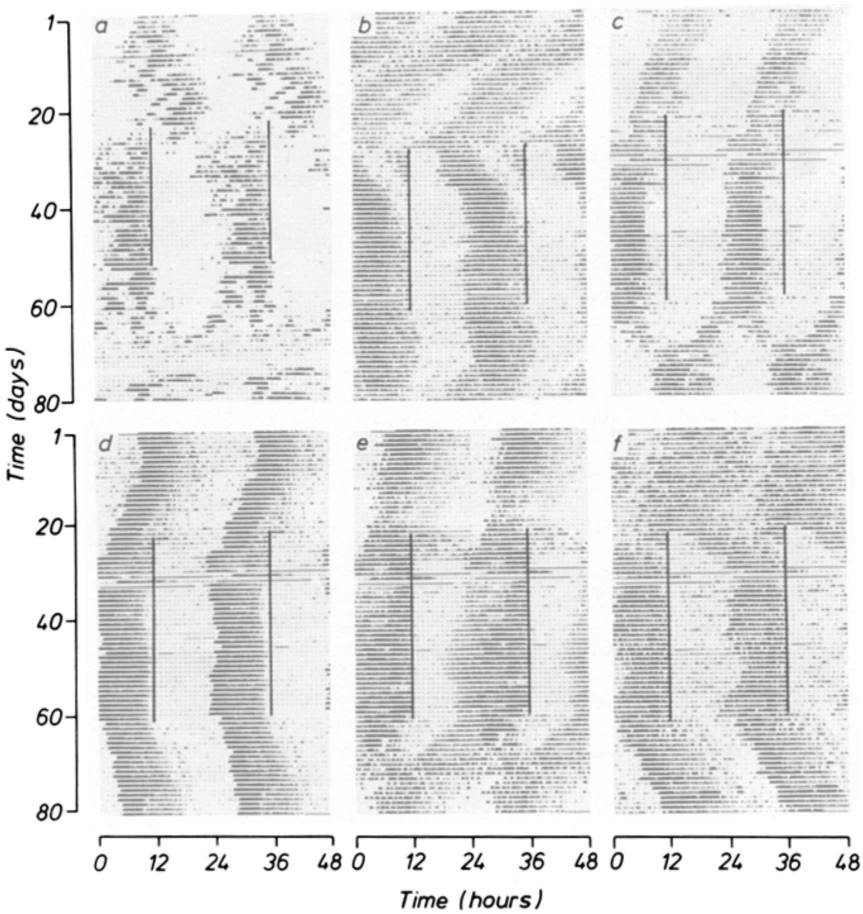


**Fig. 2 a–f.** Activity recordings of 6 pinealectomized starlings maintained in DD (**a**) or continuous dim light (**b** to **f**) which were injected with melatonin at the time of day connected by the vertical lines. For further explanations see Figure 1

the activity of 21 of the 22 melatonin-treated birds became synchronized with the 24-h rhythm of melatonin injection (Figs. 1, 2). As a rule, steady state synchronization was achieved only after a long series of transient cycles. In the synchronized state, activity time preceded the time of melatonin injection in all cases and the main portion of rest time followed it. In about 70% of the birds, activity ceased immediately after the melatonin injection (Figs. 1 b, 2 a, b, e, f). In the remainder, activity ended a few hours before injection (Fig. 2 c, d), in one case with remarkably little day to day variability (Fig. 2 c). When melatonin injections were discontinued, locomotor activity rhythms were freerunning again with periods differing from 24 h. In some cases, the steady state freerun was preceded by a "splitting" into two activity components (Fig. 2 a, c, e).

Apart from their synchronizing effects, melatonin injections in most birds had a direct inhibitory effect on locomotor activity. Although steady state synchronization was achieved usually only after many transient cycles, activity was inhibited or drastically reduced during the hours following injections from the first day of treatment on. This direct effect of melatonin is particularly conspicuous in Figures 1 a, b and 2 b, c, f.

## 4. Discussion

The results show that daily intramuscular injections of plain sesame oil in pinealectomized European starlings had little or no effect on locomotor activity, and synchronized the circadian activity rhythm in only 1 of 10 birds. In contrast, injections of the same amount of sesame-oil containing 50 μg of melatonin synchronized activity in 21 out of 22 birds. In the synchronized state the phase relationship between the time of injection and the locomotor activity rhythm was such that the main portion of the rest time followed the time of melatonin administration. This suggests a similar phase relationship between the rhythm of locomotor activity and melatonin concentration as has been found in intact birds. In chickens pineal and plasma concentrations of melatonin are high during rest time and low during activity time (Ralph, 1976, for review). Hence these findings are consistent with the hypothesis that the circadian rhythm of melatonin concentration provides an internal synchronizing agent affecting directly one or several oscillators to which locomotor activity is coupled.

However, at least one other explanation is also possible. As shown in Figures 1 and 2, melatonin, apart from its synchronizing action, also apparently exerts a direct effect on the activity-rest cycle. Injections of melatonin are followed by an immediate reduction of locomotor activity. This observation is consistent with results obtained from other species indicating that exogenous melatonin may depress locomotor activity and even have sedative or sleep inducing effects (Antón-Tay et al., 1971; Binkley, 1974; Byrne, 1970; Hendel and Turek, 1978; Hishikawa et al., 1969; Marczynsk et al., 1964; Reiss et al., 1963; Wong and Whiteside, 1968). It seems possible, therefore, that in the present experiment melatonin may have primarily exerted a direct effect on the pattern of sleep and wakefulness and that synchronization of the circadian rhythm resulted from the altered activity-rest cycle. This alternative hypothesis of an indirect synchronizing effect of melatonin due to its sleep-inducing action is testable because it predicts that synchronization should be possible with other sleep-inducing drugs as well.

When melatonin injections were discontinued, the activity of several birds separated into two components which temporarily freeran with different periods. A similar "splitting" of activity rhythms has previously been described for several vertebrate species (e.g. Hoffmann, 1971; Pittendrigh and Daan, 1976), including the starling (Gwinner, 1974). It is generally considered to reflect the uncoupling of two (or two groups of) circadian oscillators, each controlling a particular portion of locomotor activity. The occurrence of "splitting" in pinealectomized starlings, therefore, supports an earlier hypothesis (Gwinner, 1978) according to which the pineal acts on a system of at least two circadian oscillators controlling locomotor activity.

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