Die Vogelwarte 34, 1988: 276–280

Aus dem Max-Planck-Institut für Verhaltensphysiologie

Melatonin Plasma Profiles and Photoperiodic Responses in the European Starling

Von J. P. Dittami and D. Janik

In general, there are two major functions which have been attributed to the pineal in vertebrates. One is the coupling of environmental information to the physiology of an individual. This is best exemplified by the role of melatonin in the measurement of photoperiod in mammals (i.e. REITER et al. 1976, TAMARKIN et al. 1976, 1977 BITTMAN et al. 1983, GOLDMAN et al. 1984, BUBENIK 1986, POULTON et al. 1987). The other is the organization of circadian locomotor activity which has been demonstrated in a few bird species (rev. MENAKER & BINKLEY 1981). Although the relationship between these two functions would seem inherent, the circadian time measuring system ist not always involved in photoperiodic measurement (rev. in FOLLETT & FOLLET 1981). Indeed, the role of the pineal in the circadian organization of mammals is presumed to be negligible (rev. MENAKER & BINKLEY 1981) inspite of its importance in photoperiodic measurement. In addition, although the pineal is generally involved in the circadian system of birds, its role in photoperiodic reactions has not been supported by experimental results up to now (GWINNER et al. 1981, GWINNER & DITTAMI 1982). There appears to be a functional difference between birds and mammals. One problem, however, in documenting this difference is the discrepancy in experimental approaches. In mammals there has been an emphasis on determining daily circulating profiles of plasma melatonin concentrations and the effects of their manipulations with regard to photoperiodic responses (i.e. literature above and ROLLAG et al. 1978). There is little of this kind of information available in birds outside of descriptions of differing melatonin profiles in different photoperiods (LIOU et al. 1987, SHUENN et al. 1987).

Using a strongly photoperiodic species, the European starling, we therefore attempted (1) to produce a photoperiodic response by manipulating melatonin profiles when birds go from a photorefractory to a photostimulatory situation.

Methods

Two experiments were done with adult European Starlings which had been caught in Mannheim and held in outdoor aviaries in Andechs where food and water were given ad libitum.

In experiment one two groups of starlings (4 females and 1 male in each) were placed in constant condition chambers in July 1986. At this time the birds were photorefractory. The photoperiod in the chambers was set at LD 18:6 with light onset at 4:00 am. To avoid possible seasonal effects, the birds were kept in these conditions until January 1987. A time when free-living birds are known to have broken photorefractoriness while the birds in this experimental setup had not due to their long-day treatment (rev. in GWINNER 1986). From January 7 to February 12 the experimental birds were given an oral dose of melatonin (4 mg in 0.1 ml water, injected into mouth with a 1 ml syringe) daily at 15:00. This mimicked the melatonin profile of a day with a short photoperiod (Figure 1). The doses of melatonin were prepared before the experiment by dissolving 0.8 g in 2 ml ethanol and then diluting it to the proper concentration. 1 ml aliquots were separated and frozen at −20 °C until shortly before administration. The control group received water alone. All birds were laparotomized on January 5, bill color was estimated on a scale of 1 to 5 (black, non-reproductive as 1, yellow as 5). After melatonin treatment, the birds were left in the same photoperiodic conditions until March 5 (22 days after termination of treatment) when both control and experimental groups were reexamined. In order to analyze the effect of the oral dose of melatonin blood samples were taken from five birds at various intervals after application (3, 9, 16, 20 and 25 hours). This was carried out with the experimental birds in march 1987.

In experiment two we examined the naturally occurring melatonin profiles of aviary starlings in two situations: in spring (27–29 April 1988) when the birds were photosensitive and in summer (11–13 August 1988)

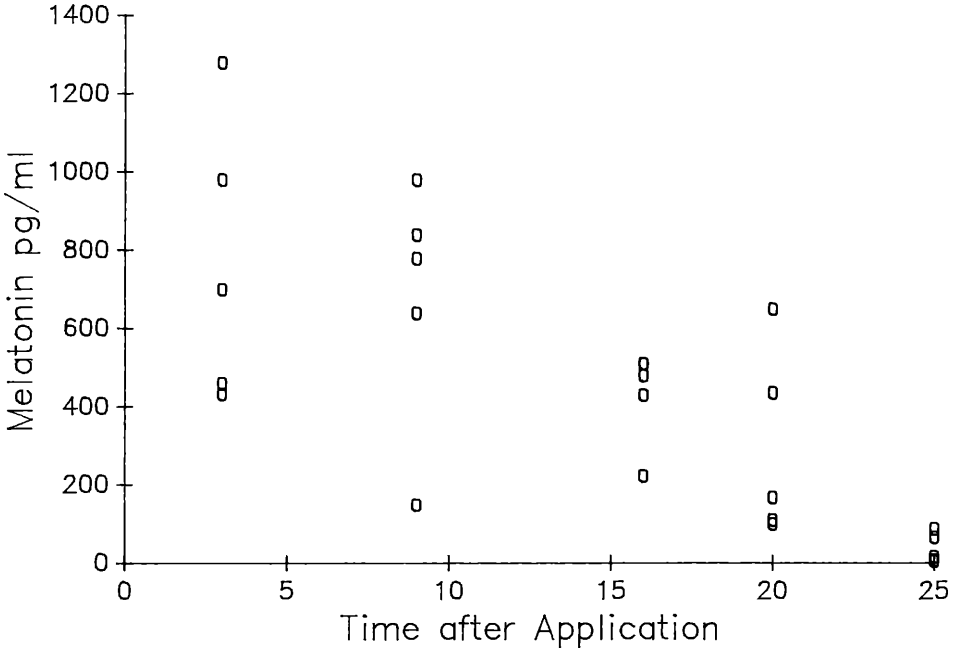

Fig. 1: Melatonin plasma concentrations 3, 9, 16, 20 and 25 hours after a 4 mg oral application.
Abb. 1: Plasmakonzentrationen von Melatonin 3, 9, 16, 20 und 25 Stunden nach oraler Applikation.

when they were photorefractory. Four aviary birds were captured and blood samples were collected at the following times and in the given sequence: 18:00, 21:00, 6:00, 9:00, 12:00, 15:00, 0:00, 3:00 and 5:00. No repeated samples were taken from an individual bird. Immediately before each blood sampling the light intensity in the aviary was measured with a luxmeter.

Melatonin was measured according to the method of FRASER et al. 1983 with the modifications given in BELDHIUS et al. 1988. LH was measured according to the method of SHARP et al. 1987.

Results and Discussion

Oral application of melatonin proved to be an effective means of prolonging its nocturnal peak (Fig. 1). Plasma levels were elevated up to 20 hours after application. Maximal values were about double those found in untreated starlings (Figure 3 and BELDHIUS et al. 1988). The results of experiment one are shown in Table 1. After the six-week melatonin treatment the birds remained exposed to long days (LD 18:6) for three weeks. The results show that no gonadal development resulted, the LH plasma titers remained basal and no coloration of the bill occurred. If the birds had broken photorefractoriness one would have expected some gonadal growth in the three weeks (GWINNER & GÄNSHIRT 1982, GWINNER & WOZNIAK 1982).

Prolonging the nocturnal melatonin peak in this manner then does not break photorefractoriness in starlings indicating that the pineal is not involved in this photoperiodic process. GWINNER et al. (1981) came to the same conclusion on the basis of published data on pinealectomy and the photoperiodic control of reproduction. There is then an inherent difference in the function of the pineal between birds (in particular starlings) and mammals where photoperiodic reactions can be manipulated by application of melatonin (i.e. REITER et al. 1976, TAMARKIN et al. 1976, 1977, BITTMAN et al. 1983, GOLDMAN et al. 1984, BUBENIK 1986, POULTON et al. 1987).

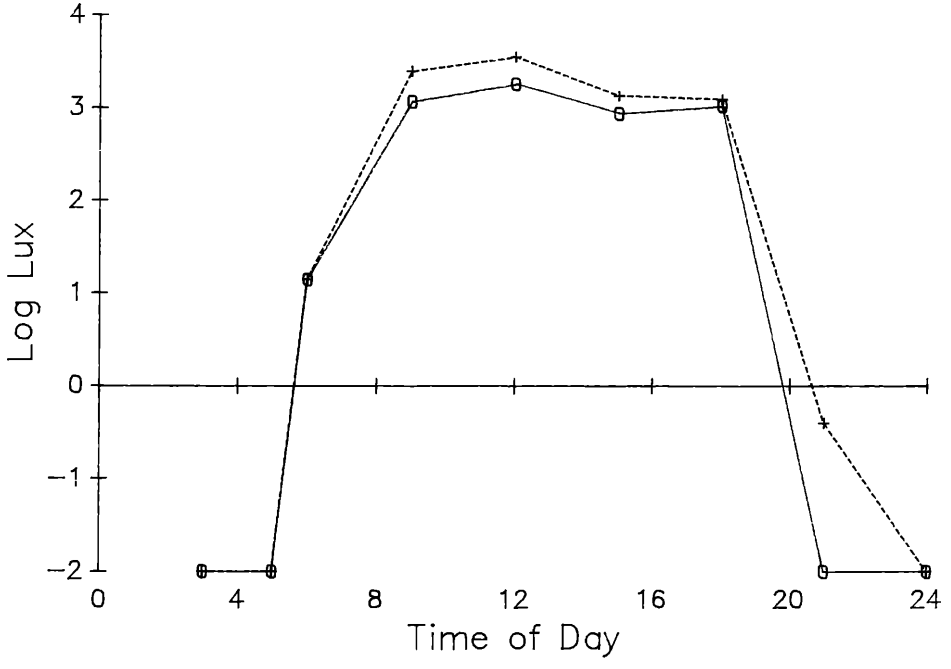

Fig. 2: Measured light intensities at the sampling times used to produce the melatonin profiles in Fig. 3. Circles represent April data, crosses August data.

Abb. 2: Gemessene Lichtintensität zum Zeitpunkt der Blutprobenentnahme (Melatonininhalt wird in Abb. 3 gezeigt). Kreise repräsentieren Messungen im April, Kreuze repräsentieren Messungen im August.

In the second experiment, we investigated whether there was a naturally occurring difference melatonin profile of photosensitive and photorefractory birds. Days with similar photoperiods before and after the summer solstice were chosen. As shown in Figure 2, changes in light intensity were similar for the April and the August data. At 21:00 the data did differ due to the fact that it rained in April and was clear in August. As a result the August 21:00 reading was higher.

Examining the melatonin data, one can see that there were no differences in the amplitude of the melatonin profiles in a photosensitive and photorefractory situation. There were slight differences in the phase and duration of the melatonin peak. The 18:00 measurements in April were all higher than those in August. The same was true at 21:00, with one point overlap. If the results could be substantiated as independent of the differences in light intensity it would mean a long melatonin peak in the inductive phase and a short one in the photorefractory phase, which is not easily interpreted.

One can then conclude that the output of melatonin in starlings is most probably not involved in the gonadal photoperiodic responses. It is not possible to manipulate photorefractoriness by the application of melatonin. The differences also in the output of melatonin between photosensitive and photorefractory birds in the same light-dark conditions are not unequivocal. Although one would have expected this from starlings on the basis of data from pinealectomy and testicular cycles (Gwinner et al. 1981, Gwinner and Dittami 1980, 1982), these results reaffirm the hypothesis using methods similar to those which have been applied to mammals.

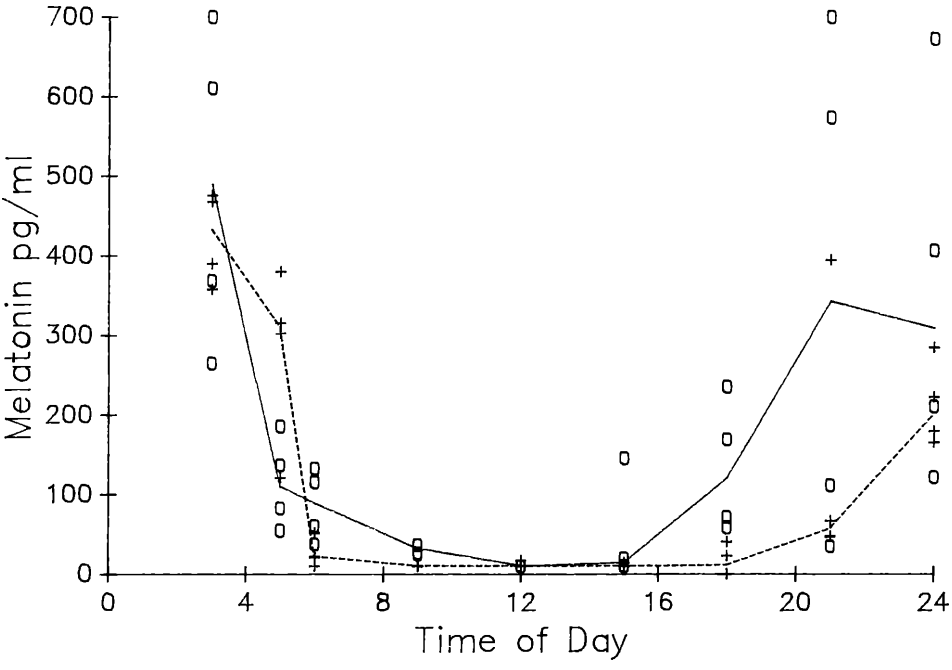

Fig. 3: Plasma concentrations of melatonin (medians and individual values) with respect to time of day. Crosses and dashed line represent data from August in the photorefractory phase, open circles and solid line represent data from April, during the inductive phase of gonadal development.

Abb. 3: Plasmakonzentrationen von Melatonin (Einzelmessungen mit Medianen) hinsichtlich der Tageszeit. Offene Kreise und durchgezogene Linien zeigen Daten der Gonadenentwicklung im April (induktive Phase), Kreuze und gestrichelte Linien zeigen Daten vom August (photorefraktäre Phase).

Summary

The hypothesis that melatonin is involved in photoperiodic responses in birds was investigated in the European starling. In one experiment photorefractory birds under long days were given an oral dose of melatonin which produced a prolonged peak in its plasma titers. After a six-week treatment the birds were kept without melatonin application for another three weeks in the same photoperiod. At that time, no changes in reproductive parameters were found (gonadal size, bill color, LH plasma concentrations). In a second experiment, the naturally occurring plasma profiles of melatonin were investigated in April during the photoinductive phase and in August during the photorefractory phases of the gonadal cycle. No amplitudinal differences were found in the melatonin cycles. The phase and duration of the melatonin peak differed slightly.

Table 1: Reproductive parameters in starlings in LD 20:4 before and after melatonin application.
Tab. 1: Fortpflanzungsparameter vor und nach Melatoninzugabe bei Staren unter LD 20:4)

	Controls		Experimentals	
	before	after	before	after
LH ng/ml	0.23±0.5	0.34±0.5	0.15±0.3	0.27±0.3
Bill color	1.0	1.0	1.0	1.0
Gonads	undeveloped	undeveloped	undeveloped	undeveloped

Zusammenfassung

Plasmakonzentration von Melatonin und photoperiodische Reaktionen beim Star

Die Hypothese, daß Melatonin photoperiodische Reaktionen hervorruft, wurde beim europäischen Star untersucht. In einem Experiment wurde, unter Langtagbedingung gehaltenen photorefraktären Vögeln, oral eine Dosis Melatonin gegeben, welche einen verlängerten Gipfel im Plasmatiter hervorrief. Nach einer sechswöchigen Behandlung wurden die Vögel drei Wochen lang ohne Melatoningabe unter denselben Bedingungen gehalten. Zu diesem Zeitpunkt haben keinerlei Änderungen ihrer reproduktiven Parameter (Gonadengröße, Schnabelfarbe, LH Plasmakonzentration) stattgefunden.

In einem 2. Experiment wurde das natürlich vorkommende Plasmaprofil während der photoinduktiven Phase im April, und während der photorefraktären Phase der Gonadenentwicklung im August untersucht. Es wurden keinerlei Unterschiede in der Amplitude des Melatoninzyklus gefunden. Bei Phase und Dauer des Melatoninhöhepunktes gab es leichte Unterschiede.

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