Borrower: PSM

Lending String: *NHM,DRB,VTU,TFW,MDY

Patron: Chabot, Chris

Journal Title: Journal of comparative physiology. A, Sensory, neural, and behavioral physiology.

Volume: 170 Issue: 5
Month/Year: 1992 Pages: 615-622

Article Author: MENAKER, Chabot;

Article Title: EFFECTS OF PHYSIOLOGICAL CYCLES OF INFUSED MELATONIN ON CIRCADIAN RHYTHMICITY IN PIGEONS

Imprint: Berlin ; New York ; Springer Verlag, [c1]

ILL Number: 62248123

Call #: 010002 6 10

Location: 010002

ARIEL
Charge
Maxcost: 25.00IFM

Shipping Address:
Plymouth State University
Lamson Library/ILL
Plymouth, NH 03264

Fax:
ARIEL: 158.136.80.183
Odyssey: 206.107.43.55
Effects of physiological cycles of infused melatonin on circadian rhythmicity in pigeons

Christopher C. Chabot* and Michael Menaker

Biology Department, University of Virginia, Charlottesville, VA 22903, USA

Accepted February 12, 1992

Summary. The role of the hormone melatonin in the circadian system of pigeons (Columba livia) was investigated. Using an automatic infusion system, melatonin at physiological levels was delivered for 10 h each day to cannulated, pinealectomized (P-X) pigeons in constant darkness. These cyclic infusions of melatonin entrained feeding rhythms in P-X pigeons while vehicle infusions were ineffective entraining agents. When the retinae of P-X pigeons were removed (E-X), feeding rhythms were abolished in constant darkness. When cyclic melatonin infusions were delivered to these birds (E-X and P-X), feeding rhythmicity was restored whereas vehicle infusions alone did not restore rhythmicity. When melatonin infusions were terminated in E-X/P-X pigeons, feeding rhythms persisted for several days but eventually decayed. Blood melatonin levels were measured in both P-X and E-X/P-X birds infused cyclically with exogenous melatonin and were found to be within the physiological range both in level and pattern. These results strongly suggest that endogenous melatonin, released by the pineal gland and the retinae, regulates the timing of feeding rhythms by entraining other oscillators in the circadian system of the pigeon.

Key words: Melatonin – Circadian rhythms – Infusions – Behavior

Introduction

Rhythmic, daily fluctuations of blood melatonin levels have been demonstrated in a number of avian species in both light:dark (LD) cycles and in constant conditions (Norris 1981; Cassone and Menaker 1984; Underwood et al. 1984; Foà and Menaker 1988). Significantly, a stable phase relationship between peak melatonin blood levels and peak locomotor activity exists in birds in both LD and in free-running conditions (Norris 1981; Oshima et al. 1987). Removal of endogenous sources of rhythmic melatonin, i.e. the retinae and/or the pineal gland abolishes locomotor rhythmicity in a number of avian species (Gaston 1971; Gaston and Menaker 1968; Ebihara et al. 1984). The implantation of melatonin-filled capsules abolishes or affects locomotor rhythmicity in house sparrows (Turek et al. 1976), Java sparrows (Ebihara and Kawamura 1981) and suppresses locomotor rhythms in pigeons (Chabot 1990). In addition, daily melatonin injections can entrain the perch hopping rhythm in starlings (Gwinner and Benzinger 1978). Collectively, these data suggest that endogenous melatonin is involved in the regulation of circadian locomotor rhythms in birds (Cassone and Menaker 1984). On the other hand, there is as yet no direct demonstration that physiological levels of melatonin presented in naturally occurring temporal patterns are sufficient to regulate circadian rhythmicity.

In the present study we sought such direct evidence; pinealectomized (P-X) homing pigeons were used to test the hypothesis that rhythmic infusions of physiological amounts of melatonin can entrain and/or restore behavioral rhythms in pigeons. Since P-X pigeons retain measurable rhythms of circulating melatonin (Foà and Menaker 1988) experiments were designed to override the influence of this rhythm on circadian rhythms of feeding using exogenously infused melatonin. In addition, P-X/bilaterally enucleated (E-X) pigeons, which lack rhythms of circulating melatonin and do not express behavioral circadian rhythms (Ebihara et al. 1984; Foà and Menaker 1988), were used to test the hypothesis that behavioral rhythmicity can be restored with physiological infusions of melatonin delivered in normal temporal patterns.
Effects of physiological cycles of infused melatonin on circadian rhythmicity in pigeons

Christopher C. Chabot* and Michael Menaker
Biology Department, University of Virginia, Charlottesville, VA 22903, USA

Accepted February 12, 1992

Summary. The role of the hormone melatonin in the circadian system of pigeons (Columbia livia) was investigated. Using an automatic infusion system, melatonin at physiological levels was delivered for 10 h each day to cannulated, pinealectomized (P-X) pigeons in constant darkness. These cyclic infusions of melatonin entrained feeding rhythms in P-X pigeons while vehicle infusions were ineffective entraining agents. When the retinae of P-X pigeons were removed (E-X), feeding rhythms were abolished in constant darkness. When cyclic melatonin infusions were delivered to these birds (E-X and P-X), feeding rhythmicity was restored whereas vehicle infusions alone did not restore rhythmicity. When melatonin infusions were terminated in E-X/P-X pigeons, feeding rhythms persisted for several days but eventually decayed. Blood melatonin levels were measured in both P-X and E-X/P-X birds infused cyclically with exogenous melatonin and were found to be within the physiological range both in level and pattern. These results strongly suggest that endogenous melatonin, released by the pineal gland and the retinæ, regulates the timing of feeding rhythms by entraining other oscillators in the circadian system of the pigeon.

Key words: Melatonin – Circadian rhythms – Infusions – Behavior

Introduction

Rhythmic, daily fluctuations of blood melatonin levels have been demonstrated in a number of avian species in both light:dark (LD) cycles and in constant condi-

tions (Norris 1981; Cassone and Menaker 1984; Underwood et al. 1984; Foa and Menaker 1988). Significantly, a stable phase relationship between peak melatonin blood levels and peak locomotor activity exists in birds in both LD and in free-running conditions (Norris 1981; Oshima et al. 1987). Removal of endogenous sources of rhythmic melatonin, i.e. the retinæ and/or the pineal gland abolishes locomotor rhythmicity in a number of avian species (Gaston 1971; Gaston and Menaker 1968; Ebihara et al. 1984). The implantation of melatonin-filled capsules abolishes or affects locomotor rhythmicity in house sparrows (Turek et al. 1976), Java sparrows (Ebihara and Kawamura 1981) and suppresses locomotor rhythms in pigeons (Chabot 1990). In addition, daily melatonin injections can entrain the perch hopping rhythm in starlings (Gwinner and Benzing 1978). Collectively, these data suggest that endogenous melatonin is involved in the regulation of circadian locomotor rhythms in birds (Cassone and Menaker 1984). On the other hand, there is as yet no direct demonstration that physiological levels of melatonin presented in naturally occurring temporal patterns are sufficient to regulate circadian rhythmicity.

In the present study we sought such direct evidence; pinealectomized (P-X) homing pigeons were used to test the hypothesis that rhythmic infusions of physiological amounts of melatonin can entrain and/or restore behavioral rhythms in pigeons. Since P-X pigeons retain measurable rhythms of circulating melatonin (Foa and Menaker 1988) experiments were designed to override the influence of this rhythm on circadian rhythms of feeding using exogenously infused melatonin. In addition, P-X/bilaterally enucleated (E-X) pigeons, which lack rhythms of circulating melatonin and do not express behavioral circadian rhythms (Ebihara et al. 1984; Foa and Menaker 1988), were used to test the hypothesis that behavioral rhythmicity can be restored with physiological infusions of melatonin delivered in normal temporal patterns.

---

Abbreviations: P-X pinealectomized; E-X bilaterally enucleated; T period of infusion cycle; LD light:dark cycle; DD constant darkness

* Current address and to whom offprint requests should be sent: Zoology Department, Miami University, Oxford, OH 45056, USA
Materials and methods

Experimental birds and housing conditions

The homing pigeons (Columbia livia: males and females, 400-600 g) used in this experiment were maintained indoors under an LD cycle of 12 h light: 12 h dark prior to experimentation. Pigeons were obtained from suppliers in Waco, TX (Louis Niconia) and Eugene, OR (University of Oregon). During experimentation, pigeons were individually housed in wire bottom cages (35 cm x 38 cm x 26 cm) suspended over a pan filled with bedding (Bed-O-Cob. The Andersons, Maumee, OH) within light-tight, wooden boxes in a temperature controlled (23°C) dark room. White noise (93 dB) was continuously present (Menaker and Eskin 1966). Food and water were available ad libitum at opposite ends of the cages and were replaced at approximately biweekly intervals. An infrared viewer (FJW Optical Systems, Elgin, IL) was used when pigeons were held in constant darkness (DD). Bedding was replaced at approximately biweekly intervals. Pigeons were always allowed at least 10 days in a 12:12 LD cycle when first placed in the cage. Feeding activity was monitored with an infrared emitter-detector pair mounted across a food access hole. Behavioral data were collected on an event recorder (Esterline-Angus, Indianapolis, IN) and later on a computer data acquisition system (Data Quest, Data-Sciences, Inc., Roseville, MN; EZ Paste software, M. Vogelbaum, Charlottesville, VA).

Surgeries

P-X and retina removal (E-X). Pigeons were P-X as previously described (Ebihara et al. 1984; Chabot 1990). For removal of the retinas, P-X pigeons were removed from their recording cages and anesthetized (60 mg/kg Ketamine followed by 10 mg/kg Nembutal). Local anesthetic (lidocaine) was applied to the eyes and the eyelid corners surgically extended. A frontal section was then cut from the eye removing the lens, iris and a ring of sclera. The vitreous was removed with absorbent cotton swabs and the retina was gently teased away from the underlying pigment epithelium. A pair of iridectomy scissors was used to detach the retina at the pecten and at the optic nerve. After surgery the inside of the eye was inspected to verify that the retina had been completely removed. Often some of the pigment epithelium was removed as well. The amount of pigment epithelium removed varied with the individual (10-90%). The cavity of the eye was packed with Gel foam (Upjohn, Kalamazoo, MI) and anesthetic ointment (gentamicin sulfate). The eyelids were sutured shut and gentamicin sulfate was applied externally. Pigeons were always allowed to recover on a heating pad before being returned to their cages in DD.

Cannulations. Cannula assemblies for delivery of melatonin (Sigma) in vehicle or vehicle alone were prepared ahead of time as follows: a small square (1.5 x 1.5 cm) of Marlex mesh (Bard Cardiovascular, Billerica, MA) was fitted over the stem of a small animal anchor button (Harvard Apparatus, South Natick, MA) and attached to the anchor button with silk sutures. A stainless steel tether was then attached to the anchor button and a cannula (tygon tubing, i.d. = 0.02 in, o.d. = 0.06) threaded through both the button and tether. Both the tether and cannula were then attached to a small animal fluid swivel (Harvard Apparatus).

Intact or P-X pigeons held in LD cycles were anesthetized as above. Local anesthetic (lidocaine HCl) was injected subcutaneously in the inter-scapular region and feathers were plucked in an approximately 2.5 cm area. A 1 cm incision was then made in the center of the plucked area and a subcutaneous space was created for the cannula and anchor button by blunt dissection. The anchor button and cannula were inserted through the incision and the incision was closed with sutures. The anchor button was then firmly attached to the pigeon with two sutures through both the Marlex mesh and the dermis. E-X/P-X pigeons were cannulated in a similar manner at the same time as E-X was performed. For a more complete description of the procedures see Chabot (1990).

Infusions

P-X pigeons. Infusions of either vehicle (phosphate buffered saline) or melatonin dissolved in vehicle into P-X pigeons was accomplished in the following manner: cannulated P-X pigeons, entrained to an LD cycle, were put in DD. On the first day in DD, and each day thereafter, the birds received a 10 h continuous infusion of vehicle or melatonin/vehicle solution. The infusion cycle began either 6 h before or 6 h after the onset of behavioral activity (subjective dawn). Infusions were controlled by a timer adjusted to have a period shorter than, equal to, or longer than 24 h (T= 23.67 - 24.77). The melatonin dosages infused (0.46, 0.7, 0.93, 1.86 and 3.72 μg/h) were controlled by adjusting either the concentration of the melatonin solution (between 1.5 - 3.0 x 10^7 g/l) or by adjusting the pump rate between 22 and 124 μl/h. Seventeen pigeons were infused with melatonin; 13 were infused with more than one timing pattern or dose, but no pigeon was infused with more than 3 different regimes. Infusion of vehicle alone was employed as a control procedure: in 6 of 22 trials vehicle alone was infused prior to melatonin infusion; in the remaining 16 trials vehicle alone was infused after melatonin infusion. Control pigeons receiving vehicle only were infused at the same rates as those receiving melatonin. Since none of the pigeons in either of the two control groups were affected by infusion of vehicle alone, the data were pooled. Overall, 12 birds received vehicle only infusions; while half of these pigeons were infused with more than one timing pattern or dose, only one pigeon was infused with as many as 4.

E-X/P-X pigeons. Cannulations and infusions were performed as described above for P-X pigeons except that, during infusions, the vehicle and melatonin/vehicle infusions were delivered at a single rate (31 μl/h for 10 h/timing cycle; dose = 0.93 μg melatonin/h). Infusions were controlled by two timers adjusted to have periods longer than 24 h only (24.51 and 24.65 h).

Blood samples

Blood samples were collected from individually housed intact, P-X and P-X/melatonin infused pigeons by brachial vein puncture as previously described (Foai and Menaker 1988). Pigeons were perfused with the aid of an intra-red viewer after birds had been in DD for at least 24 h. A series of 4 blood samples was drawn in a 24 h period (one every 6 h) and at least one week elapsed between each series of samples. Blood samples were centrifuged at 4°C for 15 min at 2900 g. One hundred μl serum samples were then aliquotted into borosilicate glass tubes and stored at -70°C until extraction and radioimmunoassay for melatonin.

Extraction, radioimmunoassay and validation

Melatonin in the serum and plasma samples was extracted with chloroform within two weeks of sampling and radioimmunoassay for melatonin was performed on the extracted samples as previously described (Foai and Menaker 1988). Known quantities of melatonin in phosphate buffered saline were also extracted and/or assayed and these values were used to correct for extraction efficiency and as inter-assay controls. The average extraction efficiency (10 extractions) was 82%. Intra-assay coefficient of variation (CV) for melatonin standards (40 pg/100 μl) in phosphate buffered saline was 7% (10 assays). The inter-assay CV for the melatonin standards was 20% and the limit of detection was 31 pg/ml (for 100 μl samples). The values for 20%, 50%, and 80% binding on standard curves were 164±122, 336±23, and 69±5 pg/ml respectively.
This assay (R1055) was previously validated for specificity of pigeon serum melatonin using high performance liquid chromatography and for parallelism of inhibition curves of pigeon serum (Foà and Menaker 1988).

Data analysis

P-X records. Behavioral records were used in the determination of entrainment only if the pigeons were allowed to free-run after the infusion cycle. By this criterion, the behavioral results from several (n=8) pigeons receiving melatonin infusions were excluded since, although their behaviors were synchronized to the infusion cycle, entrainment was not demonstrated (i.e., no free-run). The periods of free-running rhythmicity after entrainment were calculated by drawing a best eye-fit line through the offsets of feeding behavior and calculating the slope of the line. The phase angles of entrainment to melatonin infusion T cycles were determined from feeding records of pigeons that entrained clearly for at least 10 days. To determine phase angles, a best eye-fit line was drawn through the offsets of the last 10 days of entrainment. The time differential between this behavioral offset and the onset of melatonin infusion was recorded. Blood melatonin levels of P-X birds were included in the analysis only if the behavior was rhythmic enough to allow measurement of behavioral offset (22/24 trials; n=17). Three out of 32 infused pigeons had blood melatonin levels that indicated they were not receiving exogenous melatonin. These were not used in the analysis.

E-X/P-X records. When behavioral rhythmicity was clear by both visual inspection and periodogram analysis the period and phase of feeding rhythms were calculated by drawing a best eye-fit line through the feeding activity offsets. Chi-Square periodogram analyses (Sokolove and Bushell 1978) were performed using a moving window (10 days/window) procedure on all segments (P-X/E-X, P-X/E-X/vehicle infused and P-X/E-X/melatonin infused) of the behavioral records. The significance of differences between means was determined using Student's t test (P<0.05).

Results

Intact, P-X and P-X infused pigeons

Melatonin infusion. Representative feeding records from P-X pigeons entrained to LD cycles and then subjected to melatonin infusion T cycles in DD are presented in Fig. 1. Circadian rhythmicity, as measured by feeding activity, synchronized to daily infusions of melatonin delivered cyclically in the circadian range. When the melatonin infusions were terminated the feeding activity free-run with periods that differed from those of the previous melatonin infusion cycle (Fig. 1A) even when vehicle infusion was continued with the same period (Fig. 1B, C). Entrainment was seen in all 29 trials in which pigeons were given melatonin infusions followed by vehicle infusion or no infusion. Several melatonin doses (0.46 µg, 2.67 µg/h) and T cycles (between 23.67 and 24.72 h) were all able to entrain or synchronize feeding activities. The average phase angle of entrainment (length of time between feeding offset and melatonin infusion onset) in different melatonin T cycles, pooled irrespective of dose, was \(-1.63 \pm 0.22\) h (T<24 h, n=3), \(-0.14 \pm 0.43\) (T=24, n=7) and 2.53 \pm 0.44 h (T>24 h, n=11). Although these values are all significantly differ-
ent, the biological significance of the actual values may be diluted by the fact that the three groups were clustered irrespective of dose. Although we did not conduct controlled experiments to test for after-effects, the free-running periods of the behavioral rhythms did not appear to be consistently related to the period (T cycle) of the previous melatonin infusion. A typical feeding record of a pigeon which received vehicle infusion on the first day in DD is presented in Fig. 1C. Feeding activity did not synchronize to vehicle infusions whether the infusions were delivered to the pigeon on the first day in DD (Fig. 1C, top box) or several weeks later (Fig. 1C, bottom box) after entrainment to melatonin infusions (Fig. 1C, middle box). Indeed vehicle infusion was never an effective entraining agent.

**Blood melatonin levels.** Representative melatonin profiles of P-X and P-X/melatonin infused blood samples from individual pigeons and averaged intact levels are plotted versus circadian time (0600 = behavioral activity onset) in Fig. 2A–C. All of the profiles exhibited rhythmic changes in absolute melatonin levels over the course of a 24 h period. In the cases shown, P-X reduced the amplitude of the melatonin rhythm and melatonin infusion restored it to an amplitude slightly higher than intact levels (Fig. 2A–C). The peaks (maximal levels) of blood melatonin were always approximately 180° out of phase with the peaks (major bouts) of feeding activity in P-X/melatonin infused birds (Fig. 1A–C; days of blood withdrawal are indicated by arrows). The peaks of blood melatonin and feeding activity in P-X and intact birds were always antiphase as well. The average values of the blood melatonin content of samples from pineal intact, P-X and P-X/melatonin infused (0.93 µg/h) birds are presented in Fig. 2D. Thus, the same trends that were apparent in individual pigeons (Fig. 2A–C) were also apparent among the population of birds sampled.

The differences between the peak and trough values were significant for intact, P-X and P-X/melatonin infused pigeons (P < 0.001). P-X peak levels were on average lower than intact levels although the differences were not significant. Peak levels of P-X/melatonin infused individuals for this (0.93 µg/h) and higher doses (3.6 and 1.8 µg/h) were on average slightly higher than peak intact levels (P < 0.05) but were well within the physiological range of intact levels (1996–325 pg/ml; Chabot 1990). The average peak blood melatonin levels of P-X/melatonin infused birds given lower doses (0.70, 0.46 µg/h) were not significantly different than peak intact levels (data not shown). A previous study has shown that blood melatonin levels of pigeons sampled at a higher frequency (every 2 h) with the use of an indwelling cannula exhibits similar levels when intact, P-X and P-X/melatonin infused (0.46, 0.7, 0.93 µg/h; Chabot 1990). The peak levels of circulating melatonin in both intact and P-X pigeons are in close agreement with those previously published by Foã and Menaker (1988).

**Intact, E-X/P-X and E-X/P-X infused pigeons**

**Melatonin infusions.** Representative feeding records from P-X birds which were bilaterally E-X and then subjected to periodic melatonin infusion are presented in Fig. 3 (all panels). The feeding activity of P-X/E-X pigeons was arrhythmic in DD. When these birds were later subjected to rhythmic infusions of melatonin, rhythmic patterns of feeding activity were observed (Fig. 3, open box). The average period of the feeding rhythms during infusions for all birds was similar to the period of melatonin infusion [24.5 ± 0.03 h (n = 6 pigeons) vs. 24.51 (infusion period) and 24.60 ± 0.03 h (n = 8) vs. 24.65 (infusion period)]. On average the main bout of activity ended 2.1 ± 0.6 h (n = 14) before the melatonin infusions ended 2.1 ± 0.6 h (n = 14) before the melatonin infusions ended 2.1 ± 0.6 h (n = 14) before the melatonin infusions

---

**Fig. 2A–D.** Blood melatonin levels (± SEM) in intact (solid line), P-X (dashed line), and P-X/melatonin infused (dotted line) pigeons in DD. Blood was drawn at 6 h intervals for 24 h. Melatonin was delivered to P-X/melatonin infused pigeons for 10 h on the day of sampling (approximate circadian time 1900–0500). The 0400 data points have been plotted twice in each panel and the average curve for intact pigeons is repeated for reference in each panel. Blood samples are from pigeons whose feeding activity is shown in the corresponding panels in Fig. 1A–C. D is a summary graph of the average values (± SEM); intact, n = 20; P-X, n = 28; P-X/melatonin infused (0.93 µg/h), n = 11.
C.C Chabot and M. Menaker: Melatonin infusions and circadian rhythms in pigeons

Fig. 3. Representative feeding records from P-X/E-X pigeons in DD before, during (box), and after melatonin infusions (0.93 μg/h for 10 h/day). Left panel letters on the right side of this panel correspond to the section of the record subjected to periodogram analyses (Fig. 4). Infusion periods: Left, Right panels = 24.51 h; Middle panel = 24.65. Horizontal arrow day blood samples were drawn. * Day on which pigeon was bilaterally enculuated (Middle panel)

Fig. 4A-D. Chi-Square periodogram analyses of the behavioral record presented in Fig. 3 (left panel). Panels A-D correspond to sections A–D from Fig. 3 (left panel)

When the melatonin infusions were terminated, feeding rhythms persisted for several cycles (Fig. 4C); later significant rhythmicity was not observed (Fig. 4D). Significant circadian rhythmicity was not apparent in any of the 11 “free-running” P-X/E-X pigeons (before melatonin infusions). Melatonin infusions restored significant feeding rhythms to all 11 P-X/E-X birds; significant rhythmicity continued in 4 of 7 individuals that were allowed to “free-run” after the melatonin infusions. While restoration of rhythmicity occurred within a few cycles (Fig. 5) for some individuals (n = 6), rhythmic activity was not manifest for 5–16 days (Fig. 3) for others (n = 7).

While daily melatonin infusions were sufficient to restore feeding rhythmicity in P-X/E-X pigeons, daily infusions of vehicle alone were not. Representative feeding activity records from a P-X/E-X pigeon in DD which received vehicle infusions is presented in Fig. 5. As is evidenced both by visual inspection and by periodogram analysis of these data (right panels, A, B) significant behavioral rhythmicity was not observed before or during vehicle infusions. When melatonin infusions were later initiated after vehicle infusions, behavioral rhythmicity was clearly evident (Fig. 5C). Similar results were seen in all 8 other pigeons subjected to vehicle infusions followed by melatonin infusions.

P-X, P-X/E-X, and P-X/E-X/melatonin infused blood melatonin levels from individuals and grouped birds are presented in Fig. 6A–D. As was shown above in Fig. 2, blood levels of intact pigeons show clear circadian differences, the peaks of which are approximately
180° out of phase with their feeding activity peaks. The blood melatonin levels of P-X/E-X birds from samples taken immediately (within 2 cycles) after melatonin infusion was terminated were low and arrhythmic (Fig. 6A–C). When these birds were infused with melatonin (0.93 μg/h for 10 h), a blood melatonin rhythm was apparent (Fig. 6A–D). Importantly, the P-X/E-X peak melatonin levels during melatonin infusion were not higher than the peak P-X melatonin levels of these pigeons. The average blood melatonin peak values of intact \( (n = 20) \) and P-X/E-X/melatonin infused birds \( (n = 6) \) were significantly higher than the average trough values \( (P < 0.001) \). The peak levels of both intact and E-X/P-X birds \( (n = 17) \) are in close agreement with those previously reported by Foà and Menaker (1988).
Discussion

Our results demonstrate that cyclic melatonin infusions at physiological levels can 1) entrain the feeding rhythms of P-X pigeons and 2) restore behavioral circadian rhythms to pigeons rendered arrhythmic by removal of the retinae and the pineal gland. In both the P-X pigeon and E-X/P-X pigeon, infusions of melatonin, but not vehicle, were sufficient to entrain or restore behavioral rhythmicity in all cases. These results demonstrate that melatonin was the important timing signal in these infusions. Gwinner and Benzing (1978) have previously demonstrated locomotor activity entrainment of starlings to daily melatonin injections. Since blood melatonin levels either before or after injection were not determined in their study, the physiological significance of these melatonin injections cannot be determined. Similarly Oshima et al. (1989) recently presented evidence that daily melatonin injections can restore locomotor rhythms in P-X/E-X pigeons. However, control injection data were not presented and the injections induced blood melatonin levels an order of magnitude higher than normal and with grossly abnormal temporal profiles. Here we have demonstrated that the blood melatonin levels of both P-X and P-X/E-X pigeons receiving melatonin infusions were within the physiological range and temporal pattern. While melatonin has long been hypothesized to be centrally important to the control of circadian rhythmicity in birds, our results are the first direct demonstration in any avian species that physiologically significant amounts of exogenous melatonin delivered in normal temporal patterns are sufficient to entrain behavioral rhythms.

Our results confirm Foà and Menaker’s (1988) report that a large amplitude blood melatonin rhythm persists in P-X pigeons. Underwood et al. (1988, 1990) report that the eyes of Japanese quail contain circadian oscillators and Foà and Menaker (1988) have demonstrated that the eyes are the source of rhythmic blood melatonin in P-X pigeons. We did not observe dual, or especially broad, peaks of melatonin in P-X/melatonin infused pigeons – as might be expected if the exogenous (infused) and the endogenous peak (from the retinae) remained completely independent of one another. This suggests that the rhythm of retinal melatonin was entrained by the melatonin infusion cycle. Because blood was drawn from P-X/melatonin infused pigeons a few weeks after the initiation of the melatonin infusion regimen it is possible that there were dual or especially broad melatonin peaks for some portion of the period between the start of melatonin infusion and the time of our measurements. Indeed it is likely that this would occur prior to the steady state entrainment of the retinal melatonin rhythm. Furthermore, the irregularity of the behavioral rhythms occasionally observed during this time (Fig. 1 A) may have been due to this phenomenon.

In addition to demonstrating that feeding rhythms in pigeons entrain to melatonin infusion cycles our data indicate differences in the measured phase angles of entrainment to different T cycles of melatonin infusion. These results suggest that the target system on which melatonin acts in pigeons is itself an oscillator (Pittendrigh and Daan 1976). That persistence of rhythmicity in some E-X/P-X individuals after melatonin infusion was stopped (Fig. 3) also indicates that melatonin entrains an oscillator(s) in E-X/P-X pigeons. Previous results from Ebihara et al. (1984) provided evidence that E-X/P-X pigeons also entrain to LD cycles. When these LD entrained birds were subsequently exposed to constant conditions, significant behavioral rhythmicity persisted for several cycles. Thus, our present results and those from Ebihara et al. (1984) demonstrate the presence of (an) oscillator(s) outside of the eyes and the pineal gland which is entrainable by melatonin and light. Results from hypothalamic lesion studies (Ebihara et al. 1987) have not yet enabled identification of this oscillator. However, a synthesis of the results from a number of experiments on avian species has led Cassone and Menaker (1984) to suggest that the avian suprachiasmatic nuclei are damped oscillators that are dependent on rhythmic input, perhaps from melatonin (or light), to maintain behavioral rhythmicity. This suggestion is still consistent with the known facts and given the role of the suprachiasmatic nuclei in mammals (Ralph et al. 1990) is eminently reasonable.

While our results concerning the abolition of behavioral and blood melatonin rhythms are similar to previously published reports (Ebihara et al. 1984; Foà and Menaker 1988), there is a potentially significant methodological difference between our study and previous studies. While in removing the retina, Ebihara et al. (1984) and Foà and Menaker (1988) removed the whole eye (and inevitably most of the Harderian gland as well) we removed only tissues inside the sclera. This suggests that the tissue responsible for the maintenance of locomotor rhythmicity is inside the eye and is probably the retina since this tissue has been shown to contain high levels of N-acetyl transferase activity as well as high concentrations of melatonin in birds (Hamm et al. 1983; Underwood et al. 1988) and in some other vertebrates such as Xenopus laevis (Cahill et al. 1991).

Although the Harderian gland has been previously suggested as a source of melatonin in the pigeon on the basis of a tissue content study (Vakkuri et al. 1984), our method of E-X, which left the Harderian gland intact, abolished both blood melatonin and behavioral rhythms. Therefore, the Harderian glands do not appear to be capable of maintaining blood melatonin or feeding rhythms in pigeons in the absence of the retinae and the pineal gland.

The results reported here strengthen the argument that melatonin is a critical messenger in the avian circadian system. The direct demonstration that circadian behavior of pigeons can be entrained or restored by physiological amounts of melatonin infused in temporally normal patterns strongly supports the hypothesis that endogenous melatonin rhythms regulate circadian rhythms in intact pigeons and by inference in other avian species as well.

Acknowledgements. Our sincere thanks are given to Christopher Colwell and Marianna Max for many useful discussions concerning
References


Norris C (1981) Circulating levels of melatonin in the house sparrow, Passer domesticus. MA Thesis, University of Texas, Austin, Texas


