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Melatonin rhythms and pineal structure in a tropical bat, *Anoura geoffroyi*, that does not use photoperiod to regulate seasonal reproduction

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Abstract: It has been hypothesized that pineal structure and function might differ between temperate zone and tropical species of mammals because of lower amplitudes of seasonal change in photoperiod and, in some areas, less seasonal climatic variation. *Anoura geoffroyi* produce a single offspring in November or December of each year on the Caribbean island of Trinidad, at 10°N latitude in the deep tropics. Previous work has shown that this population lacks reproductive responses to photoperiod, and must be enforcing seasonal breeding using a non-photoperiodic cue. *Anoura geoffroyi* have a minute, thin, and rod-like pineal gland. Throughout much of its length, the pineal courses irregularly within the ventrolateral wall of the great cerebral vein. This intimate relationship may have functional implications. Despite having a very small pineal gland, this species produced a nocturnal rise in serum melatonin. Serum melatonin levels in most individuals were below or near undetectable levels during the light period and rose to a peak averaging 100 pg/ml in the last third of the dark period. Our results indicate that, although the pineal gland of *A. geoffroyi* is extremely small, serum melatonin levels are comparable to those of other mammals.

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Introduction

The pineal gland is an essential link in a neuroendocrine pathway that transduces seasonal changes in photoperiod into seasonal changes in physiology and behavior in many mammals [Tamarkin et al., 1985; Turek and Van Cauter, 1994; Bronson and Heideman, 1994]. The mammalian pineal gland receives input via a complex pathway beginning in the retina and passing successively through the retinohypothalamic tracts, the suprachiasmatic nuclei, the paraventricular nuclei, superior cervical ganglia, and adrenergic fibers of the sympathetic system to terminate in the pineal gland [reviewed by Tamarkin et al., 1985]. Besides the central innervation of the pineal gland, recent studies have implicated extracerebral parasympathetic ganglia as additional sources of pineal innervation [see review by Møller, 1992]. The pineal gland is stimulated to secrete melatonin at low levels during the day and high levels at night. Thus, the melatonin signal can convey information on daylength, and, therefore, on season as well. Seasonal changes in melatonin production regulate changes in physiology and behavior in numerous species of mammals, many of which are related to reproduction [reviewed by Bittman, 1993; Bronson and Heideman, 1994]. While melatonin affects many physiological systems in mammals [Arendt, 1992; Reiter, 1993], the transduction of seasonal photoperiod changes to seasonal physiological signals appears to be a major function of melatonin and the pineal gland in mammals.

A number of authors [Pévet and Kuyper, 1978; Pévet and Yadav, 1980; Chang et al., 1987] have suggested that pineal structure and function might differ between temperate zone and tropical species of mammals for two reasons. First, seasonal changes in daylength are much lower in amplitude in the tropics, declining to zero on the equator, resulting in a signal which must be useless on the equator as well as for some unknown distance north and south of the equator. Second, seasonal changes in climate

can be slight in some parts of the tropics, which could reduce selective pressure favoring seasonal responses in mammals. Alternatively, pineal gland structure and function might be similar in tropical and temperate mammals because of non-seasonal actions of the pineal gland and melatonin. While there is a significant literature on pineal structure in tropical mammals [Pévet and Yadav, 1980; Bhatnagar et al., 1985; Bhatnagar et al., 1986; Chang et al., 1987; Bhatnagar, 1988; Bhatnagar et al., 1990; Bhatnagar, 1992; Bhatnagar and Hilton, 1994], it does not include data on melatonin production or seasonality in tropical mammals. Thus, it is not clear how differences in the morphology of the pineal gland in tropical mammals are related to levels of melatonin production or to the use of photoperiod to regulate seasonal changes in physiology or behavior.

In this study, we examine pineal structure and melatonin production in *Anoura geoffroyi,* a seasonally breeding mammal from 10°N latitude in the deep tropics [Heideman et al., 1992], in which there appears to be no reproductive response to photoperiod [Heideman and Bronson, 1994]. The objectives of this study were, first, to determine serum melatonin levels over a 24 hr period and, second, to compare pineal structure and function in this tropical bat with published data from other tropical and temperate zone species.

Methods

Males of Geoffroy's hairy-legged, long-tongued bats, *Anoura geoffroyi* (Family Phyllostomidae) were collected from Tamana Cave (10°28′N, 61°12′E, 240 m elevation) in central Trinidad in 1990 and 1992 and brought to the University of Texas. This species feeds on nectar, pollen, soft fruit, and soft-bodied insects [Goodwin, 1946; Goodwin and Greenhall, 1961; Alvarez and Gonzalez, 1970; Howell and Burch, 1974; Sazima and Sazima, 1978; Baumgarten and Vieira, 1994]. *Anoura geoffroyi* have relatively large eyes and presumably rely heavily on vision. They are small (12–19 g), agile fliers and are capable of hovering flight. The species ranges from central Mexico south across the equator to Peru, Bolivia, and east-central Brazil [Eisenberg, 1989]. At Tamana Cave, *A. geoffroyi* roost in large groups in chambers 15–30 m from the cave entrances. In these roosts, light intensity is below 1 lux (unpublished observations, Heideman and Bronson), but light from the entrances is apparent, and the bats experience higher light intensities when circling near cave entrances at dusk and dawn [Darlington, 1970] or at other times of day.

Housing conditions in the laboratory have been described by Heideman et al. [1992] and are briefly summarized here. Bats were individually marked and placed in groups of 11–13 in light-sealed, ventilated flight cages. Each cage had a feeding area 87 cm long, 62 cm wide, and 55 cm high, lighted during part of each day, and connected at one end by an opening to a roosting area 30 cm long, 62 cm wide, and 55 cm high, which received light only through the opening to the flight area. The bats were fed a diet slightly modified [Heideman et al., 1992] from Rasweiler's [1973; diet XI] formula for maintaining phyllostomid bats. The diet was a mixture of canned peach nectar, water, sucrose, nonfat and whole milk powder, high protein baby cereal, wheat germ, and supplements of essential oils, vitamins, and minerals. The temperature and relative humidity were maintained at 27 ± 2°C and 70 ± 10%, respectively.

Light was provided by two 20 W externally ballasted fluorescent bulbs, one at the ceiling of each end of the feeding area. These provided light intensities of approximately 1,000–2,000 lux in the flight area and under 100 lux in most of the roost area. The onset and end of the light period in each cage was controlled by a Hunter timer (model 41001; Hunter Fan Co., Memphis, TN) programmable to the minute, and accurate to within 1 sec per day. The bats in this study had previously been exposed to a variety of different treatments designed to mimic potential seasonal entraining cues, including differences in food quality, humidity, light intensity, light position, and timed exposures to dim light and polarized light for a period of 6 months. All bats were then given 1 month of standard treatment and diet (see above) and a photoperiod cycle of lights on from 0400 to 1600 hr before blood samples were taken for melatonin assay.

Morphological studies

Male bats were given a lethal dose of pentobarbitol (90 mg/kg body weight, i.p.), and then perfused with either Bouin's solution [Humason, 1972] or a 3.5% glutaraldehyde and 2% paraformaldehyde solution in 0.18M sodium cacodylate buffer, pH 7.35 [Bhatnagar, 1988]. Brains were either removed and then processed for paraffin sectioning followed by staining with Gomori trichrome or hematoxylin and eosin, or the entire head was decalcified (in 125 ml formic acid, 50 g sodium citrate, 375 ml distilled water, solution changed weekly for 4 months [Bhatnagar and Kallen, 1974]) and then processed as above. Sections 6–10 μm thick were mounted serially and examined under a light microscope. The serial sections were then projected and the region encompassing the pineal was traced. From these serial tracings, Figure 1 was constructed as a three-dimensional presentation.

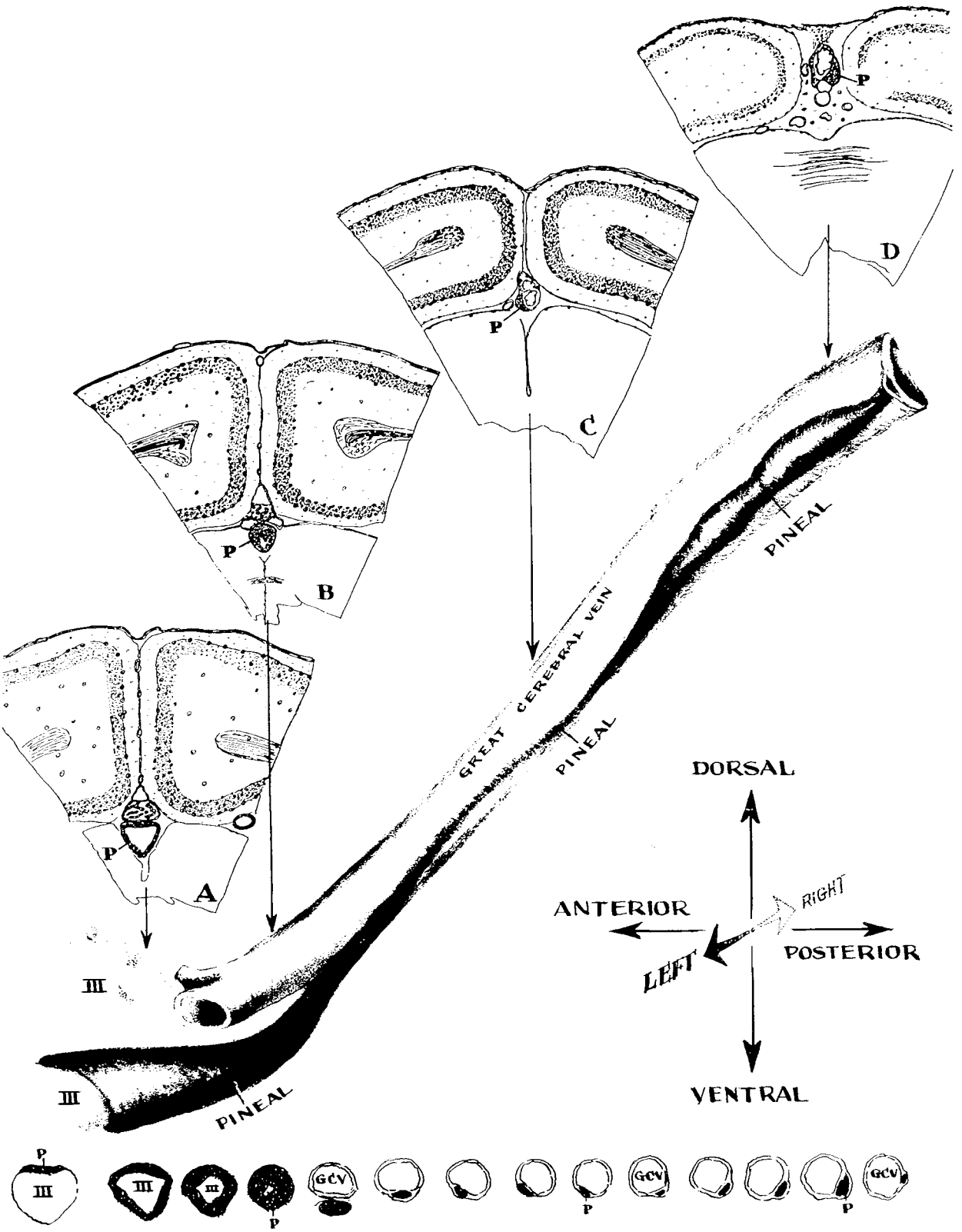

Fig. 1. Schematic drawing of the nearly 2 mm long pineal gland of a male *Anoura geoffroyi* and its relationship to the posterior aspect of the third ventricle and to the great cerebral vein (GCV). These associations are shown in the bottom panel consisting of 14 cross-sectional profiles each taken from the serial projections at approximately 126 mm intervals (see Methods). A–D represent the actual projection images of selected cross sections from the regions indicated by the arrows. Note the position of the pineal (P) to the surrounding brain structures.

Melatonin radioimmunoassay

Serum melatonin concentrations were determined with a direct radioimmunoassay (RIA) [Heideman and Bronson, 1990] adapted from one described for human plasma [Fraser et al., 1986] and later modified for use in sheep [English et al., 1986]. Briefly, the assay followed procedures outlined by S.M. Yellon (personal communication) using a Guildhay antiserum (G/S 704-6483) raised in sheep against N-acetyl-5-methoxytryptophan (obtained from J. Arendt, University of Surrey, Guildford, Surrey, UK). Melatonin was measured in 200–500 µl samples of serum from male bats. The minimal detectable amount of melatonin, defined as 2 S.D. from the buffer controls, was 3.2 pg/sample (20 pg/ml for 200 µl samples and 8 pg/ml for 500 µl samples after allowing for small losses of serum during chloroform extraction). The data presented here were obtained from a single assay with an intra-assay coefficient of variation of 10%.

The assay was validated for *Anoura geoffroyi* in three ways. First, we tested for parallelism between duplicate samples of serial dilutions from a pool of blood collected from *A. geoffroyi* at night and from serial dilutions of a standard solution of melatonin (Sigma, M-5250). The 500 µl samples from this pool (N = 10) had 27 and 33 pg of melatonin, respectively; the 250 µl samples had values of 13 and 13 pg of melatonin, respectively; and the 150 µl samples 7 and 8 pg of melatonin, respectively. Second, we tested for parallelism between serial dilutions of a pool containing mixed pineal and neural tissue collected at night from eight bats and serial dilutions of the standard solution of melatonin described above. Because the pineal of *Anoura geoffroyi* is minute (see Results), we collected pineal glands or gland fragments along with surrounding tissue. One-half of this pool of daytime pineals held 16 pg of melatonin; one-fourth of the pool held 7 pg of melatonin; one-eighth of the pool held 3 pg of melatonin; and in the sample of one-sixteenth of the pool melatonin was undetectable. Third, we added small known quantities of melatonin (10 and 25 pg) to aliquots from a pool of serum from *Anoura* collected during the day; assays of these samples gave measurements that were 70% and 94% of expected values, respectively.

In pilot work, blood samples were collected either from the major vein at the anterior edge of the propatagial membrane of the wing [Kunz and Nagy, 1988] or from the trunk following rapid decapitation. Both methods gave comparable values of serum melatonin in both day and night samples, but collection from the vein required pooling of the serum from 100 µl samples of plasma collected over a number of nights or from a number of bats. In order to obtain a 24 hr profile of serum melatonin values, trunk blood samples were collected at 0200, 0600, 1000, 1400, 1800, and 2200 hr (N = 6, 6, 8, 5, 5, and 4, respectively). The samples were collected on two consecutive nights, with blood from two to four individuals collected at each time (see above) over the two nights. During the dark period collections, the bats were exposed to approximately 1–3 min of indirect dim red light from a 15 W incandescent bulb (covered by a metal lamp shade) immediately before blood collection. All samples were allowed to clot for 15–60 min at 4°C and then centrifuged at 3,000 rpm for 15 min, following which serum was drawn off and stored at –80°C until assayed.

Statistical analysis

The analysis of melatonin data was carried out using the SuperANOVA (version 1.1, Abacus Concepts, Berkeley, CA) and Resampling Statistics (version 4.0; Resampling Stats, Inc., Arlington, VA) programs for Macintosh computers, except that Bartlett's test [Sokal and Rohlf, 1981] for homogeneity of variances was conducted using a hand calculator. Only the results of a randomization test equivalent of analysis of variance [Manly, 1991], with 10,000 iterations, are presented in the text because sample variances were highly heterogenous and appeared non-normal, and transformations did not fully solve these problems. The randomization test was based on an F-statistic-equivalent using the sum of squared deviations within and between groups [Manly, 1991]. Randomization, ANOVA, and non-parametric tests gave qualitatively similar results, except that the resampling method provided *P*-values approximately tenfold higher (i.e., closer to non-significance, though still significant) than the parametric or non-parametric methods.

The results of a planned comparison of differences between day and night conducted using a randomization test are presented in the text. This test compared the size of the difference between the means of night and day samples in the observed data with 10,000 iterations in which the data points were randomly reassigned to "day" and "night" groups [Manly, 1991]. In this case, the test results were almost identical to those obtained with a parametric planned contrast comparison in an analysis of variance.

Results

Pineal gland

The pineal in *Anoura geoffroyi* extends about 1.9 mm from its deep origin to termination close to the surface of the brain (Fig. 1). Because of the coni-

cal shape at its base with subsequent attenuation, the *Anoura* pineal is classified as Aβγ type [following Vollrath, 1979, and Bhatnagar et al., 1986]. Throughout much of its length the pineal courses irregularly within the ventrolateral wall of the great cerebral vein (Figs. 1, 2). The pineal begins just posterior to the habenular nuclei and almost immediately expands to envelop a tiny posterior extension of the third ventricle, taking on the appearance of a hollow cone whose base is 0.25 mm in diameter (Fig. 2A). At the termination of the ventricular lumen the pineal tissue coalesces into a solid structure (Fig. 2B), which begins to attenuate, turn in a superior direction, and merge with the adventitia of the ventrolateral surface of the great cerebral vein (Fig. 2C). The pineal tissue continues in this position, occupying more of the wall until it is separated from the lumen only by the endothelium and con-

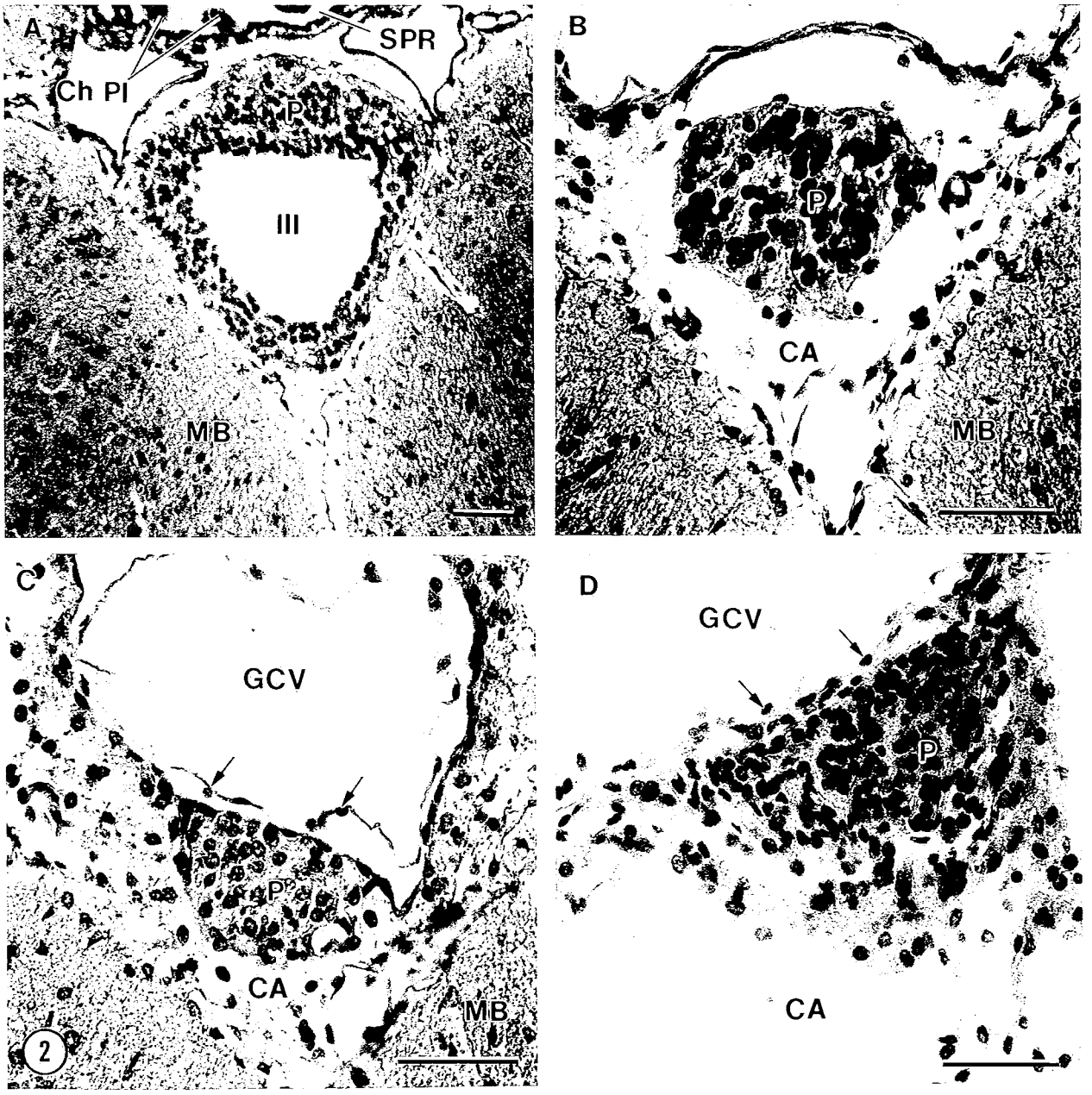

Fig. 2. Micrographs through the pineal region of *Anoura geoffroyi*. x.s. **A:** Pineal (P) surrounding the posterior projection of the third ventricle (III). ×180. **B:** Pineal integrated into the ventral aspect of the great cerebral vein (GCV). ×350. **C:** Pineal separated from the lumen of the GCV only by the endothelium (arrows). ×370. **D:** The pineal has expanded and moved laterally within the vessel wall as it approaches the brain surface. ×350. CA, cisterna ambience; Ch. Pl., choroid plexus; MB, midbrain; SPR, suprapineal recess. Scale bars = 50 mm.

nective tissue elements (Figs. 2C, 2D). The delicate capsule surrounding the pineal fuses with the connective tissue, filling the cisterna ambiens around the great cerebral vein. As the cords of pinealocytes continue to ascend in the vessel wall, the pineal tissue again expands, giving rise to a second enlargement (Fig. 2D), which then attenuates suddenly until it disappears in a more superficial region (Fig. 1). The parenchymal cells of the pineal appear to be arranged in loose cords, and they contain spherical nuclei (Fig. 2). The organ is not extensively vascularized, but does contain some intrapineal neurons. One small ganglion of fewer than a dozen cell bodies was observed in close proximity to the pineal, but probably not within its capsule. Intrapineal or meningeal concretions (acervuli) were not observed.

Melatonin levels

Mean serum melatonin levels ranged from undetectable to 38 pg/ml in samples collected during the light period, and from 52 to 100 pg/ml in samples collected during the dark period (Fig. 3). There were significant differences in melatonin levels across the sample times ($P < 0.05$), with significantly higher levels of melatonin during the night ($P < 0.001$).

Discussion

Anoura geoffroyi have a very small pineal gland relative to body mass. *Anoura geoffroyi* are comparable in size to the big brown bat, *Eptesicus fuscus* (14–22 g body weight), yet the pineal mass appears much smaller than that of *E. fuscus* [Bhatnagar and Hilton, 1994]. The intimate relationship between the pineal and the great cerebral vein appears unique to *A. geoffroyi* and has not been reported for any other animal. Many species, such as the laboratory rat [Boeckmann, 1980], and leaf-nosed bat Hipposideros lankadiva (K P. Bhatnagar, unpublished observations) have stalked pineals. In some instances the stalk has been reported to be lacking [as in 6% of rats investigated; Boeckmann, 1980], and if present, devoid of pineal parenchymal cells. However, examination of the closely associated great cerebral vein might disclose the pineal tissue, which could be embedded in the vessel wall, as observed in *A. geoffroyi*. Careful examination of the great cerebral vein should be undertaken especially in those species for which either the entire pineal or one of its components is reported to be missing [Bhatnagar et al., 1986, p. 153].

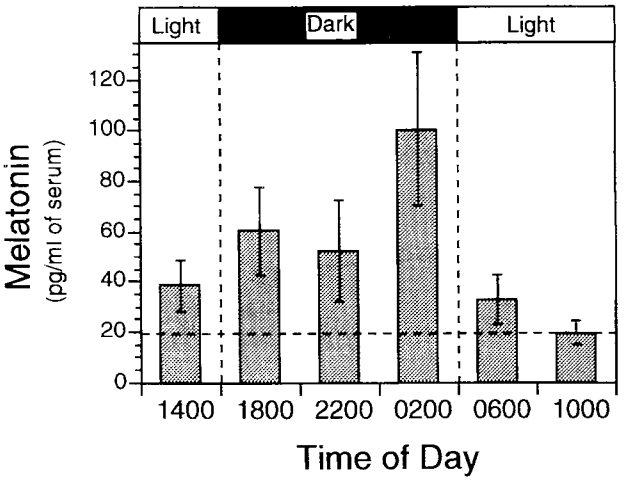

Fig. 3. Serum melatonin levels (mean ± standard error, in pg/ml) of *Anoura geoffroyi* at six times of day (see Methods for sample sizes). The dark period was from 1600 to 0400 hr. The dashed horizontal line shows the limit of detection of the assay.

In *A. geoffroyi*, endothelium alone separates the venous blood from products synthesized by the pineal. This close relationship, therefore, would seem to have functional implications. We speculate that melatonin from the pineal might be delivered efficiently to the blood across the endothelium, which may account for the robust peak of melatonin during the night.

The pineal gland of *Anoura geoffroyi* falls within the range of sizes already reported for tropical bats [Bhatnagar et al., 1986], which range from pineal glands that are missing, or minuscule, as in *A. geoffroyi*, to pineal glands that are the largest reported, relative to body mass, in any mammal [e.g., in *Dobsonia praedatrix;* Bhatnagar et al., 1990]. Our observations of the small size of the pineal gland in *A. geoffroyi* are consistent with the hypothesis that the pineal gland may be functionally less important in mammals that use non-photoperiodic cues to regulate seasonal changes in physiology. Previous work shows that *A. geoffroyi* must use non-photoperiodic cues to regulate their seasonal reproduction [Heideman and Bronson, 1994], and it is possible that these bats do not use photoperiod to regulate any seasonal changes. It is possible that the pineal gland of *A. geoffroyi* is relatively small because it serves little, if any, function. Nevertheless, these bats do produce a nocturnal rise in melatonin, whose amplitude may be sufficient to provide biologically significant physiological signals. It would be valuable to know whether tropical species with large pineal glands, such as *D. praedatrix*, use photoperiod to respond to seasonal change.

It has been suggested [Bhatnagar et al., 1990] that pineal size might be related to the degree of diurnality or, in bats, to roost location. However, Bhatnagar et al. [1990] noted that some of the bats with the largest relative pineal size roost during the

day in caves, as does *Anoura geoffroyi* with its very small pineal gland, while others with relatively large pineal glands roost in tree canopies. They concluded that relative pineal size may be unrelated to roost location or to degree of diurnality and nocturnality.

It is surprising that nocturnal levels of serum melatonin in *A. geoffroyi* are comparable to serum melatonin levels in animals with a much greater relative pineal size. Comparisons of serum melatonin levels across species are somewhat problematical because of variation among assay types, but similar assay procedures using a specific antibody tend to produce reliable and repeatable estimates of melatonin levels. With the Guildhay antiserum used in this assay, we and others find in mammals the average serum levels of melatonin levels of mammals to be in the range of 50–150 pg/ml during the nocturnal rise [Heideman and Bronson, 1990; Webley et al., 1985; English et al., 1986; Foster et al., 1989; Yellon and Longo, 1987; Yellon and Hilliker, 1994]. Night-time serum melatonin levels in *A. geoffroyi*, about 50–100 pg/ml, are well within this range. Thus, despite the small size of the pineal gland in *A. geoffroyi,* this species is able to produce a substantial night-time rise in serum melatonin.

We know of no previous report of melatonin levels in the bat suborder Microchiroptera. Our assay of serum samples from another microchiropteran bat, the big brown bat, *Eptesicus fuscus,* gave serum melatonin values similar to *A. geoffroyi*—below 20 pg/ml in a pool of day-collected serum, and 46 pg/ml in a pool of night-collected serum (Heideman, Bhatnagar, Hilton, and Bronson; unpublished data). McGuckin and Blackshaw [1992] assessed serum melatonin levels of *Pteropus poliocephalus,* a member of the other bat suborder, *Megachiroptera.* They reported serum levels under 90 pg/ml during the day and 100–300 pg/ml during the night.

Is it possible that melatonin synthesized in other organs than the pineal gland contributes to the nocturnal rise in serum melatonin in *A. geoffroyi*? The location and small size of the pineal in *A. geoffroyi* made it impossible for us to pinealectomize these animals and test for subsequent reduction or abolishment of serum melatonin levels. In many vertebrates, melatonin is reported to be produced in non-pineal tissues, such as the retina and harderian gland, but in mammals these other sources apparently contribute little or nothing to circulating melatonin [reviewed by Krause and Dubocovich, 1990]. Regardless of the source of melatonin in *A. geoffroyi,* it appears that the nocturnal rise in serum melatonin could be sufficient to serve normal functions, and is consistent with the increase in night-time melatonin synthesis in other mammals.

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Literature cited

Alvarez, T., Q. Gonzalez (1970) Analisis polinico del contenido gastrico de murcielagos glossophaginae de Mexico. An. Esc. Nac. Cienc. Biol. 18:137–165.

Arendt, J. (1992) The pineal. In: Biologic Rhythms in Clinical and Laboratory Medicine. Y. Touitou, E. Haus, eds. New York: Springer-Verlag, pp. 348–362.

Baumgarten, J.E., E.M. Vieira (1994) Reproductive seasonality and development of *Anoura geoffroyi* (Chiroptera: Phyllostomidae) in central Brazil. Mammalia 58:415–422.

Bhatnagar, K.P. (1988) Ultrastructure of the common vampire bat, *Desmodus rotundus*. Am. J. Anat. 181:163–178.

Bhatnagar, K.P. (1992) The ultrastructure of mammalian pinealocytes: a systematic investigation. Microsc. Res. Tech. 21:85–115.

Bhatnagar, K.P., N. Chang, E. Merrell (1985) Ultrastructure of the pineal organ of the Indian mouse-tailed bat, *Rhinopoma microphyllum*. Myotis 23–24:45–50.

Bhatnagar, K.P., H.D. Frahm, H. Stephan (1986) The pineal organ of bats: A comparative morphological and volumetric investigation. J. Anat. 147:143–161.

Bhatnagar, K.P., H.D. Frahm, H. Stephan (1990) The megachiropteran pineal organ: A comparative morphological and volumetric investigation with special emphasis on the remarkably large pineal of *Dobsonia praedatrix*. J. Anat. 168:143–166.

Bhatnagar, K.P., F.K. Hilton (1994) Observations on the pineal gland of the big brown bat, *Eptesicus fuscus*: Possible correlation of melanin intensification with constant darkness. Anat. Rec. 240:367–376.

Bittman, E.L. (1993) The sites and consequences of melatonin binding in mammals. Am. Zool. 33:200–211.

Boeckmann, D. (1980) Morphological investigation of the deep pineal of the rat. Cell Tissue Res. 210:283–294.

Bronson, F.H., P.D. Heideman (1994) Seasonal regulation of reproduction in mammals. In: The Physiology of Reproduction. 2nd edition. E. Knobil, J.D. Neill, eds. New York: Raven, pp. 541–584.

Chang, N., K.P. Bhatnagar, M.T. Tseng, K.B. Karim (1987) Ultrastructure of the pineal gland of the tropical bat *Rousettus leschenaulti*. Acta Anat. 128:194–203.

Darlington, J.P. (1970) Studies on the Ecology of the Tamana Caves with Special Reference to Cave-dwelling Cockroaches. Unpublished doctoral dissertation, University of West Indies, Trinidad. 167 pp.

Eisenberg, J.F. (1989) Mammals of the Neotropics, Vol. 1. The Northern Neotropics. Chicago: Chicago University Press.

English, J., A.L. Poulton, J. Arendt, A.M. Symons (1986) A comparison of the efficiency of melatonin treatments in advancing oestrus in ewes. J. Reprod. Fertil. 77:321–327.

Foster, D.L., F.J. P. Ebling, L.E. Claypool, R.I. Wood, T.E. Adel, W. Schramm (1989) Amplitude modulation of the nightly melatonin rise in the neonatal lamb and the subsequent timing of puberty. Biol. Reprod. 40:920–928.

FRASER, S., P. COWEN, M. FRANKLIN, C. FRANEY, J. ARENDT (1986) Direct radioimmunoassay for melatonin in plasma. Clin. Chem. 29:396–397.

GOODWIN, G.G. (1946) Mammals of Costa Rica. Bull. Am. Mus. Nat. Hist. 87: 275–458.

GOODWIN, G.G., A.M. GREENHALL (1961) A review of the bats of Trinidad and Tobago. Bull. Am. Mus. Nat. Hist. 122:191–301.

HEIDEMAN, P.D., F.H. BRONSON (1990) Photoperiod, melatonin secretion, and sexual maturation in a tropical rodent. Biol. Reprod. 43:745–750.

HEIDEMAN, P.D., F.H. BRONSON (1994) An endogenous circannual rhythm of reproduction in a tropical bat, *Anoura geoffroyi,* is not entrained by photoperiod. Biol. Reprod. 50:607–614.

HEIDEMAN, P.D., P. DEORAJ, F.H. BRONSON (1992) Seasonal reproduction of a tropical bat, *Anoura geoffroyi,* in relation to photoperiod. J. Reprod. Fertil. 96:765–773.

HOWELL, D.J., D. BURCH (1974) Food habits of some Costa Rican bats. Rev. Biol. Trop. 21: 281–294.

HUMASON, G.L. (1972) Animal Tissue Techniques. San Francisco: W.H. Freeman and Co.

KRAUSE, D.N., M.L. DUBOCOVICH (1990) Regulatory sites in the melatonin system of mammals. Trends Neurosci. 13:464–470.

KUNZ, T.H., K.A. NAGY (1988) Methods of energy budget analysis. In: Ecological and Behavioral Methods for the Study of Bats. T.H. Kunz, ed. Washington: Smithsonian Institution Press, pp. 277–303.

MANLY, B.F.J. (1991) Randomization and Monte Carlo Methods in Biology. London: Chapman and Hall.

MCGUCKIN, M.A., A.W. BLACKSHAW (1992) Effects of photoperiod on the reproductive physiology of male flying foxes, *Pteropus poliocephalus*. Reprod. Fertil. Dev. 4:43–53.

MØLLER, M. (1992) Fine structure of the pinealopetal innervation of the mammalian pineal gland. Microsc. Res. Tech. 21:188–204.

PÉVET, P., M.A. KUYPER (1978) The ultrastructure of pinealocytes in the golden mole (*Amblysomus hottentotus)* with special reference to the granular vesicles. Cell Tiss. Res. 191:39–56.

PÉVET, P., M.YADAV (1980) The pineal gland of equatorial mammals. I. The pinealocytes of the Malaysian rat (*Rattus sabanus)*. Cell Tiss. Res. 210:417–433.

RASWEILER, J.J. IV (1973) Care and management of the long-tongued bat, *Glossophaga soricina* (Chiroptera: Phyllostomatidae), in the laboratory, with observations on estivation induced by food deprivation. J. Mamm. 54:391–404.

REITER, R.J. (1993) A brief overview of new areas of pineal research and some old ones revisited. In: Melatonin and the Pineal Gland—From Basic Science to Clinical Applications. Y. Touitou, J. ARENDT, P. Pevet, eds. Elsevier Science Publishers, pp. 323–330.

SAZIMA, M., I. SAZIMA (1978) Bat pollination of the passion flower, *Passiflora mucronata,* in south eastern Brazil. Biotropica 10:100–109.

SOKAL, R.R., F.J. ROHLF (1981) Biometry. San Franscisco: W.H. Freeman and Company.

TAMARKIN, L., C.J. BAIRD, O.F.X. ALMEIDA (1985) Melatonin: A coordinating signal for mammalian reproduction? Science 227:714–720.

TUREK, F., W.E. VAN CAUTER (1994) Rhythms in reproduction. In: The Physiology of Reproduction, 2nd edition. E. Knobil, J.D. Neill, eds. New York: Raven, pp. 487–540.

VOLLRATH, L. (1979) Comparative morphology of the vertebrate pineal complex. Prog. Brain Res. 52:25–38.

WEBLEY, G.E., H. MEHL, K.P. WILLEY (1985) Validation of a sensitive direct assay for melatonin for investigation of circadian rhythms in different species. J. Endocrinol. 106:387–394.

YELLON, S.M., S. HILLIKER (1994) The influence of acute melatonin treatment and light on the circadian melatonin rhythm in the djungarian hamster. J. Biol. Rhythms 9:71–81.

YELLON, S.M., L.D. LONGO (1987) Melatonin rhythms in fetal and maternal circulation during pregnancy in sheep. Am. J. Physiol. 252:E799–E802.