Proc. Natl. Acad. Sci. USA
Vol. 76, No. 2, pp. 999–1003, February 1979
Physiological Sciences

The pineal gland: A pacemaker within the circadian system of the house sparrow

(circadian rhythms/birds/oscillator)

NATILLE HEADRICK ZIMMERMAN* AND MICHAEL MENAKER†‡

*Zentrum für Anatomie und Cytobiologie, Justus Liebig Universität, Aulweg 123, 6300 Giessen, West Germany; and †Department of Zoology, University of Texas, Austin, Texas 78712

Communicated by Colin S. Pittendrigh, November 20, 1978

ABSTRACT **Transplantation of pineal tissue into the anterior chamber of the eye rapidly reestablishes rhythmicity in arhythmic pinealectomized sparrows and also transfers the phase of the donor bird's rhythm to the host. Thus, the transplanted pineal does not merely permit rhythmicity to be expressed but rather transfers an oscillation that controls the remainder of the circadian system and restores the capacity for self-sustained rhythmicity. Long-term recordings, during which sparrows were exposed to various lighting conditions, demonstrate a remarkable similarity between the circadian system in normal birds and that in birds bearing pineal transplants.**

Surgical removal of the pineal organ abolishes the free-running circadian rhythms of locomotor activity and body temperature in the house sparrow (*Passer domesticus*) as well as the rhythm of migratory restlessness in the white-crowned sparrow (*Zonotrichia albicollis*) (1–4). This striking result is open to several general lines of interpretation and we have, in previous studies, attempted to discriminate among them (4–6). Because the steps between the circadian pacemaker and the overt rhythm are surely multiple and complex, an interruption at any level within this pathway may disrupt overt rhythmicity. Thus, arhythmicity produced by a surgical lesion might reflect direct effects on a pacemaker, effects on internal coupling among components of the circadian system, or effects on peripheral processes.

The pineal left *in situ* but deprived of its neural connections retains the ability to support circadian organization of locomotor activity. Chemical sympathectomy (which disrupts the only known neural input to the pineal), surgical disruption of the pineal stalk (which contains the only neural output), or a combination of these two procedures does not abolish free-running locomotor rhythmicity. These experimental results suggest that a hormonal output from the pineal couples it to the rest of the circadian system; this suggestion has been explicitly confirmed by pineal transplant experiments (6). When transplanted into the anterior chamber of the eye of a pinealectomized and therefore arhythmic sparrow, a donor pineal rapidly reestablishes circadian rhythmicity in the locomotor activity of the host bird (Fig. 1). If the eye bearing the transplanted pineal is later removed, activity becomes arhythmic.

All of the above results are compatible with either of two general hypotheses concerning the pineal's role. The gland may be secreting a substance that in some unspecified way enables a circadian pacemaker located elsewhere to modulate locomotor activity. Alternatively, the gland may contain a circadian pacemaker and its secretory output may itself confer circadian patterning on locomotor behavior. If the pineal contains a pacemaker it should be possible to demonstrate that it carries some identifiable feature of the donor's circadian rhythm with it when it is transplanted into a host bird. The experiments reported here were designed to test this possibility.

MATERIALS AND METHODS

House sparrows of both sexes were collected in Austin, TX, and maintained in outdoor aviaries. During experiments, birds were housed individually in cages within light-proof boxes in a dark room at a temperature of 23 ± 2°C. Birds were isolated from sound disturbances by random noise at 92 decibels. Food and water were available ad lib and were replaced at approximately 3-week intervals. Light for the light-dark cycles (LD) and for bright constant light (LL) was supplied by 4-W "cool white" fluorescent bulbs (Ken-Rad P4T5) that produced an intensity of 200–300 lux at the floor of the cage.

Birds were anesthetized by injections into the breast muscle of 0.1 mg of sodium pentobarbital (Nembutal) in 0.1 ml of sterile water. For details of pinealectomy and for the procedures used in pineal transplantation see ref. 7.

Forty-two donor pineals were transplanted individually into 41 pinealectomized sparrows (1 bird received two transplants and had a period of arhythmicity between removal of the first transplant and implantation of the second). To be useful in the determination of phase, rhythmicity must be established soon after the transplantation. Only those 18 cases in which rhythmicity was evident in the host bird by day 3 after transplantation have been included in this analysis. In other hosts a variable period of little activity (six cases) or arhythmicity (eight cases) preceded the development of persistent rhythmicity; these have not been included in the phase determinations. In 10 cases, persistent rhythmicity did not develop after pineal transplantation.

RESULTS

Control of Phase in the Reestablished Rhythm. Although both phase and period length of an overt rhythm reflect the properties of the underlying pacemaker, period length in *P. domesticus* is probably too labile (8) to allow meaningful comparison between the period of the donor bird and the period of the rhythm reestablished in the host. On the other hand, phase, as measured by activity onset, can be precisely controlled in donor birds by entraining them to light cycles and can be assayed with reasonable accuracy in the reestablished rhythms of host birds, provided that rhythmicity is evident within a few days of transplantation.

If pineals from donor birds held on light cycles were transplanted into arhythmic pinealectomized birds kept in constant darkness, the phase of the newly established rhythm might

---

Abbreviations: LD, light/dark cycle; LL, constant light; DD, constant dark.

‡ To whom reprint requests should be addressed.

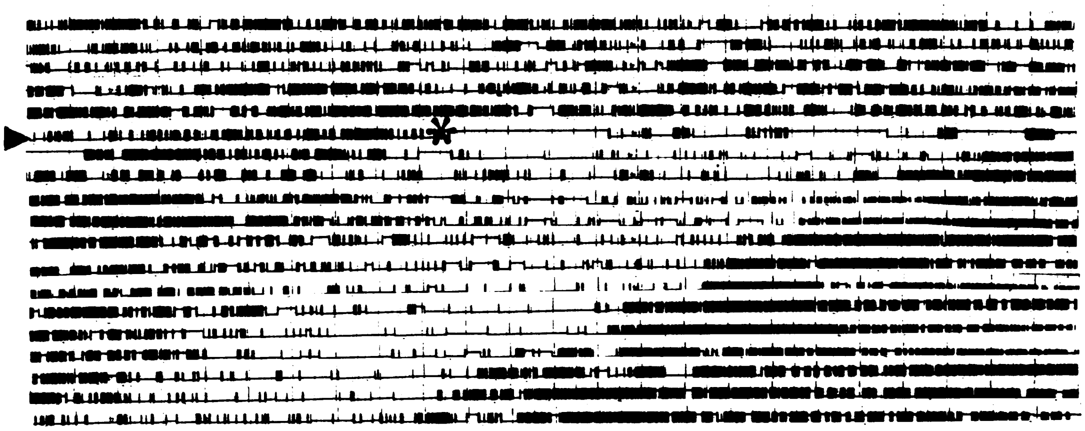

FIG. 1. Rhythmicity in pinealectomized sparrow after pineal transplantation. Day on which donor's pineal was implanted is indicated by arrowhead at left margin; the time of implantation is indicated by the asterisk. The animal was in constant darkness except for a brief exposure to light at the time of surgery. Each horizontal line represents 24 hr of locomotor activity recorded from a microswitch connected to the bird's perch. Time reads from left to right; days read from top to bottom.

behave in one of three ways. (*i*) Activity onset might always occur a given number of hours after the transplantation operation; this result would mean that a pacemaking oscillator either in the pineal or in the host bird had been reset or set in motion by the operation and would not distinguish between the two general hypotheses outlined above. (*ii*) Activity onset might occur at random times; this result would suggest that there was, in the host bird, a pacemaking oscillator that had continued its motion after pinealectomy (although no longer able to control activity) and had progressed to some phase point that could become evident only on the addition of a pineal to the system. (*iii*) Activity onset might occur at a time closely related to that of the previous activity onset of the donor bird; in that case the phase of the reestablished rhythm would have been determined by the phase of the donor bird's pineal. This could only occur if that pineal contained a pacemaking oscillator the phase of which was not greatly changed by the surgical procedure.

Donors were entrained to one of two LD 12:12 regimens; lights-on was at 0900 in one group ("LD" donors) and at 2300 for the other group ("DL" donors). In Fig. 2, the two light-dark schedules are diagrammed above the donors' activity records. The time of activity onset of the donor birds is determined by the entrainment cycle to which they are exposed (i.e., activity onset occurs at approximately the time of lights-on). In order to standardize the time of operation within the lights-on segment of each donor's cycle, all pineals were removed and transplanted between 0900 and 1100. All hosts had been previously pinealectomized, had undergone control procedures (anesthetization and brief exposure to light without tissue implantation) without initiation of rhythmicity, and were demonstrably arhythmic in constant dark (DD).

The phase of activity onset of the rhythm established by an implanted pineal approximated the phase of activity onset of the donor bird. Raw data from 2 of the 18 successful experiments are shown in Fig. 2. When the LD bird was used as a donor, activity onset of the host after transplantation occurred between 0600 and 0800 which is quite close to the donor's previous activity onset at 0900 (Fig. 2 *left*). In contrast, when the DL bird was used as a donor, activity onset (extrapolated to the first day after the transplantation) was at about 0130, very different in real time but again close to the donor's previous onset at 2300 (Fig. 2 *right*).

Activity records from the 18 successful experiments (8 using DL donors and 10 using LD donors) have been analyzed for phase of activity onset. The onsets fell into two groups that correspond to the onsets of the DL and LD donors, and there was no overlap between the two groups (Fig. 3). The phases of the rhythms produced by transplanted pineals were neither distributed at random nor determined by the time of operation but were clearly and closely related to the phases of the donor birds.

Characteristics of the Circadian System of Birds Bearing Pineal Transplants. The pineal transplant is capable of maintaining the circadian organization of locomotor activity for extended periods. Most birds remained rhythmic for as long as the transplant was left in place (5–7 months in many cases; 20 months in one case). It is therefore possible to make long-term studies of the circadian system driven by a transplanted pineal. Like normal birds, pinealectomized birds that are rhythmic because they bear a pineal transplant become arhythmic in LL and entrain to LD 12:12. Their records appear similar to the records of normal birds under these lighting conditions (Fig. 4). However, sufficient data have not yet been obtained to rule out the existence of quantitative differences.

During the first 2–3 months in DD, normal sparrows exhibit different patterns in their free-running periods (8). In DD the patterns of birds bearing pineal transplants fell within the range of those of normal birds. Twenty-nine birds remained undisturbed in DD for at least the first 2 months after implantation of a pineal that induced rhythmicity. The free-running periods in 8 of these birds were immediately and permanently long (τ = 24.5–25 hr), in 1 the period was short and continued so for 12 months (τ = 23–23.5 hr), in 14 they were initially short but lengthened to about 24.5–25 hr (Fig. 4), and in 6 they remained near 24 hr.

In about 20% of all birds with successful pineal transplants, the free running in DD was interrupted by temporary "arhythmicity" that lasted for as little as 10 and as many as 100 days before activity again became rhythmic. During this time the rhythm was clearly disrupted; however, the activity was not always evenly distributed during the 24 hr. We no not yet have much information about this state into which host birds sometimes lapse but it may well be different from the arhythmicity induced by pineal removal.

Normal birds free-running in DD can be phase shifted by exposure to single light pulses. Eskin (8) has constructed a fairly extensive phase-response curve for the phase-shifting effect of 6-hr light pulses on *P. domesticus*. In Fig. 5, Eskin's phase-response curve for normal birds has been reproduced and phase shifts produced by 6-hr light pulses in birds bearing pineal transplants have been added. The responses of transplanted

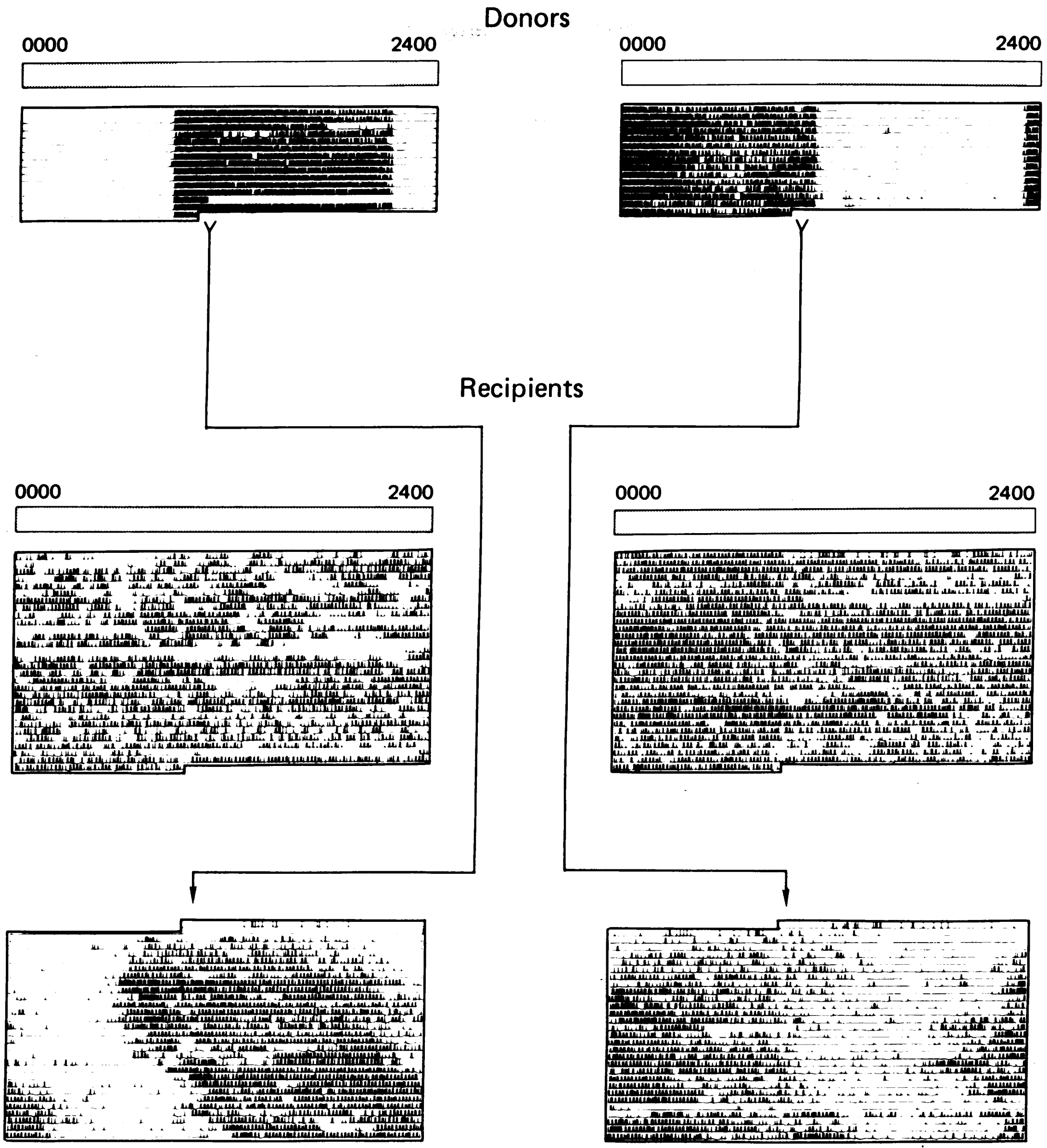

FIG. 2. Experimental design for transplantation of pineals from donors on different entrainment schedules. The donors' LD cycles [LD (*Left*) and DL (*Right*)] are represented at the top and their activity records are plotted just beneath. The hosts were in constant dark (DD) except for the brief light exposure at the time of operation. The record of each host, although continuous in time, has been separated to emphasize the day and time of pineal transplantation.

birds to 6-hr light pulses are indistinguishable from those of normal birds. All phase shifts produced in transplanted birds were in the direction and within the amplitude range of those of normal birds. The fact that phase shifting appears normal is a strong indication that, when transplantation of a pineal gland into the anterior chamber of the eye induces rhythmicity, it restores the circadian system to its prelesion state.

DISCUSSION

The circadian system of the house sparrow can be experimentally disrupted by removal of the pineal gland and can be reconstituted by transplantation of a donor's pineal into the anterior chamber of the eye (5, 6). Two important facts have been established about the reconstituted system: (*i*) its phase is derived from the implanted gland and not from either the phase of surgery or the properties of the nonpineal components of the system; and (*ii*) the reconstituted system is able to maintain its organization for long periods of time and responds in apparently normal fashion to environmental light regimens. The facts to date are compatible with, and in fact strongly support, a simple model of the physiological organization of the sparrow's circadian system that we have previously constructed (5). If these interpretations are correct, then the sparrow pineal, in and of itself, is a driving oscillator (or pacemaker) that imposes circadian temporal order on a set of subsystems within the bird. This does not imply that the pineal functions independently of the rest of the bird. It almost certainly receives both tonic influences and information about the state of its dependent subsystems. What is implied is that the pineal contains machinery that transforms appropriate noncircadian inputs into circadian outputs. Such transformations are effected by almost all multicellular organisms but they have not as yet been demonstrated to occur in any other defined structure of a vertebrate and in very few invertebrates (9).

We have recently reviewed what is known of the organization of the circadian system of the house sparrow (4, 5, 9). Here,

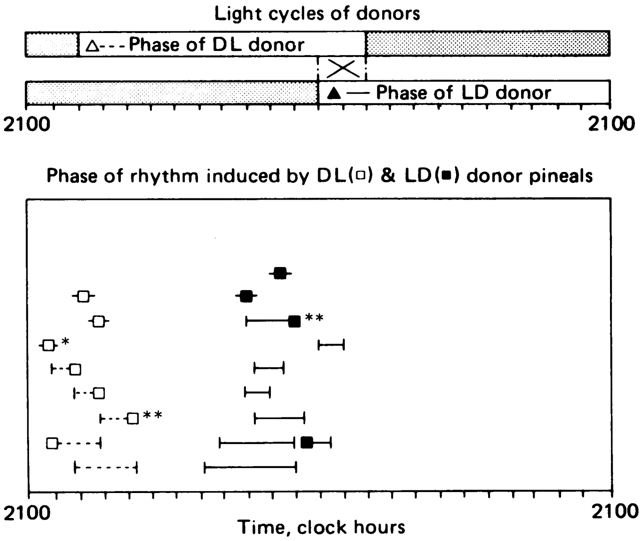

FIG. 3. Phases of activity onsets after pineal transplantations from donors on different entrainment schedules. For better visualization of the relationship between activity onset and the donor's light cycle (diagrammed at the top of the figure) the time axis has been drawn from 2100 to 2100. All surgery was done during the 2-hr overlap of the light periods. The symbols indicate variability in the precision with which activity onset can be estimated. When activity onset was clearly defined, a square indicates the best estimate of its phase; a horizontal line indicates the limits of uncertainty. A horizontal line alone indicates an onset that is within that range but cannot be determined more precisely. The experiment shown in Fig. 1 is designated by *; the two experiments shown in Fig. 2 are designated by **.

it seems appropriate only to assess the implications of and the questions raised by our current understanding without restating the detailed facts.

If the sparrow's pineal is indeed a self-contained clock, then upon establishment of the proper conditions it should be possible to assay and analyze its circadian rhythm in organ culture. Binkley *et al.* (10) have demonstrated that the chicken pineal retains time-keeping ability for at least 12 hr after it is removed and placed into organ culture. Recently, Kasal *et al.* (11) have been able to obtain four circadian cycles of *N*-acetyltransferase activity from organ-cultured chicken pineal glands.

We have as yet no information bearing on a central question that must be resolved at the cellular/biochemical level: Is the circadian organization of the pineal a cellular property? If every cell is a circadian oscillator, then we must try to understand first how the circadian oscillation is generated within the cell and second how the cells are coupled together and how their hormonal outputs are synchronized. Alternatively, it is possible that no cell is by itself a circadian oscillator but rather that the circadian oscillation of the gland is an emergent property of cellular interaction. In that case the mechanism of coupling among cells becomes not a secondary question but a primary one, central to an understanding of the generation of circadian rhythmicity. The biochemical basis of the rhythm is thus to be sought either within individual cells of the gland or between and among them. In any case the machinery must involve the regulation of a hormonal output. If, as seems plausible, that hormonal output is melatonin (12) then biochemical handles on the regulation of its synthesis are already available (13–15). The more closely the control of its synthesis is related to the generation of rhythmicity, the easier will be the unravelling of the clock mechanism at the biochemical level.

We have previously discussed questions about environmental input to the pineal and about how the pineal oscillator may be related to the other components of the sparrow's circadian

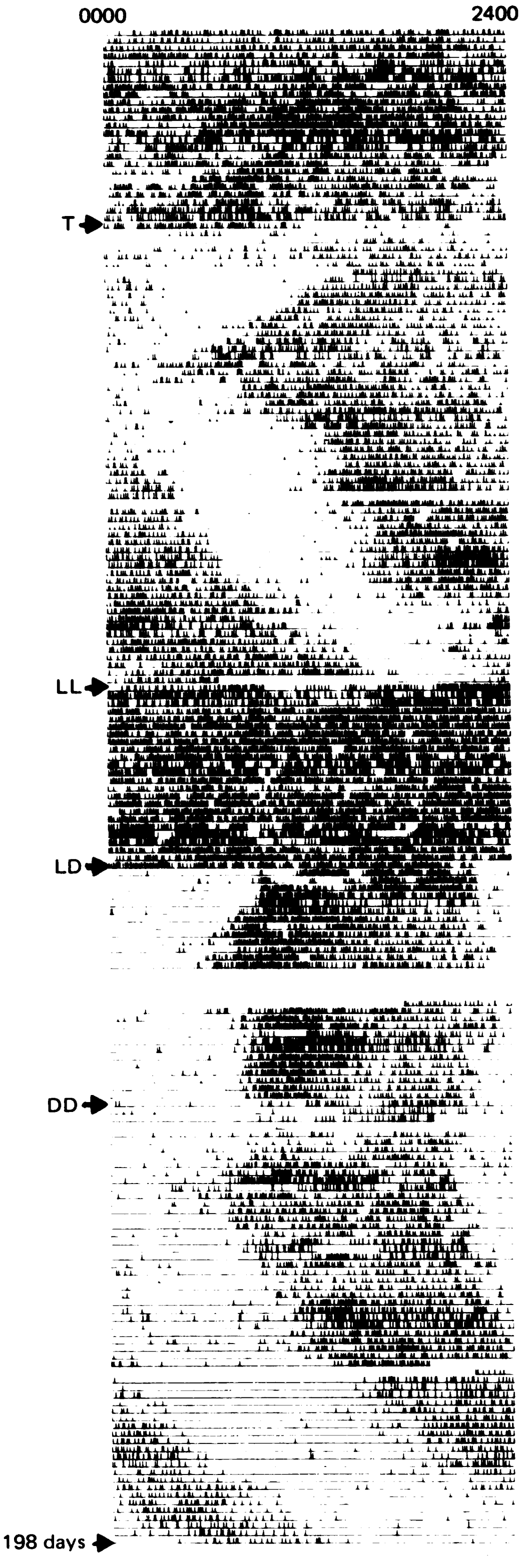

FIG. 4. Activity record from a sparrow bearing a pineal transplant and exposed to different environmental lighting conditions. The bird had been pinealectomized prior to the beginning of the record. Except for the brief light exposure at the time of pineal transplantation (T) the sparrow was in DD until exposed to constant bright light (LL). After 23 days in LL, the bird was exposed to 30 days of LD 12:12 and then returned to DD for the remainder of the record.

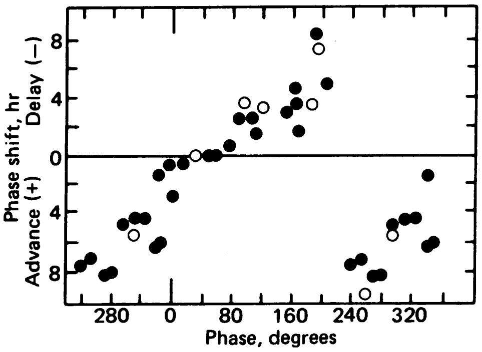

FIG. 5. Phase-response curve (amplitude and direction of phase shift produced by a 6-hr light pulse as a function of the phase of its presentation in the bird's circadian day) of normal birds (●) and birds bearing a pineal transplant (○). Data points for normal sparrows are as determined by Eskin (8). The phase of activity onset has been arbitrarily set at 0°. Each hour of the circadian cycle is 15°. Therefore, a light pulse at 150° would come 10 hr after the bird's activity onset. The shift in phase that follows a light pulse is measured in hours and is plotted as either an advance (+) or a delay (−).

system (4–6, 9). The work reported here serves most importantly to document the role of the pineal as a driving oscillator within what is clearly a multicomponent system. However, the present work does raise particular and important questions about inputs and outputs to the transplanted pineal. Is the gland obtaining light information, as implied by the "normal" responses to light pulses of the reconstituted system? Is the pineal itself light sensitive? If not, how does light information reach it? The transplanted gland is almost certainly reinnervated by sympathetic fibers. What role, if any, do such fibers play in its circadian functioning? By what route does the transplanted pineal exert its hormonal effects? Although the "obvious" answer is via the circulatory system, we cannot exclude axoplasmic transport, especially because the hypothalamus may well contain other components of the circadian system (4) and retinohypothalamic tracts have been described in birds (16).

Although the available comparative information is still sparse, if pineal function is viewed in evolutionary perspective, the beginnings of a pattern emerge. In vertebrate phylogeny, the pineal's earliest function may have been as photoreceptor specialized to detect duration and intensity and perhaps even wavelength and direction of the ambient light field. That kind of photic information is of particular importance in the regulation of daily and seasonal rhythmicity. From its original role as supplier of photic information, the degree to which the pineal subsequently became more deeply incorporated into the circadian systems of vertebrates has varied from group to group. It may have achieved its most central role, that of driving oscillator, in birds but there is now strong evidence for its importance in the circadian system of lizards (17); it is involved in time-compensated orientation in amphibians (18); and its well-documented involvement in mammalian reproduction is clearest in seasonal breeders (19, 20) that are photoperiodically regulated, probably via circadian mechanisms (21).

This work was supported in part by National Institutes of Health Grant HD-03803 and National Institute of Mental Health Training Grant MH-12476.

1. Gaston, S. & Menaker, M. (1968) *Science* **160,** 1125–1127.
2. Binkley, S., Kluth, E. & Menaker, M. (1971) *Science* **174,** 311–314.
3. McMillan, J. P. (1972) *J. Comp. Physiol.* **79,** 105–112.
4. Takahashi, J. S. & Menaker, M. (1979) in *Proceedings of the XVII International Ornithological Congress*, in press.
5. Menaker, M. & Zimmerman, N. H. (1976) *Am. Zool.* **16,** 45–55.
6. Zimmerman, N. H. & Menaker, M. (1975) *Science* **190,** 477–479.
7. Zimmerman, N. H. (1976) Dissertation (University of Texas, Austin, TX).
8. Eskin, A. (1971) in *Biochronometry*, ed. Menaker, M. (National Academy of Sciences, Washington, DC), pp. 58–78.
9. Menaker, M., Takahashi, J. S. & Eskin, A. (1978) *Annu. Rev. Physiol.* **40,** 501–526.
10. Binkley, S., Riebman, J. B. & Reilly, K. B. (1977) *Science* **197,** 1181–1183.
11. Kasal, C. A., Menaker, M. & Perez-Polo, R. (1979) *Science*, in press.
12. Turek, F. W., McMillan, J. P. & Menaker, M. (1976) *Science* **194,** 1441–1443.
13. Binkley, S. (1976) *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **35,** 2347–2352.
14. Binkley, S., MacBride, S. E., Klein, D. C. & Ralph, C. L. (1975) *Endocrinology* **96,** 848–853.
15. Ralph, C., Binkley, S., MacBride, S. & Klein, D. (1975) *Endocrinology* **97,** 1373–1378.
16. Hartwig, H. G. (1974) *Cell Tissue Res.* **153,** 89–99.
17. Underwood, H. (1977) *Science* **195,** 587–589.
18. Adler, K. (1976) *Photochem. Photobiol.* **23,** 275–298.
19. Reiter, R. & Sorrentino, S. (1970) *Am. Zool.* **10,** 247–258.
20. Turek, F. W., Desjardins, C. & Menaker, M. (1976) *Biol. Reprod.* **15,** 94–97.
21. Elliot, J. A. (1976) *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **25,** 2339–2346.