Circadian Organization in Lizards: The Role of the Pineal Organ

Abstract. *After pinealectomy, the iguanid lizard* Sceloporus olivaceus *exhibits a "splitting" of the circadian activity rhythm into two components, marked changes in the period of the rhythm, or arrhythmicity. These effects are consistent with either of two hypotheses: (i) The pineal organ is a coupling device between circadian oscillators in a multioscillator system, or (ii) the pineal organ is itself a master oscillator, which entrains other circadian oscillators and imposes its frequency upon them.*

The term "biological clock" describes the internal timekeeping machinery that is responsible for the approximately daily (circadian) changes in an organism's biochemistry, physiology, and behavior. A multicellular organism exhibits many circadian rhythms; but all, under natural environmental conditions, show determinant phase relationships with the others. Such temporal organization could be accomplished if the organism's circadian rhythms were either driven by a single biological clock (or driving oscillator) or by two or more oscillators if these oscillators were tightly coupled to one another. Until recently, information on the anatomical location or physiological basis of the clock controlling circadian rhythms in vertebrates was conspicuously lacking. However, recent studies have implicated the suprachiasmatic region of the hypothalamus of mammals (*1*) and the pineal organ of birds (*2, 3*) as part of the timekeeping machinery.

I now report that the pineal organ is also an important component of the circadian system in lizards. Many lizard species possess two members of the pineal system, the pineal organ and the parietal eye. Both of these photosensitive organs are derived embryologically as evaginations of the roof of the diencephalon (*4*). In addition, both are also active sites for the synthesis of chemicals such as the biogenic amines serotonin and melatonin (*5*). The parietal eye can have a well-developed nerve which contains both afferent and efferent fibers (*6*). The afferent fibers carry photic information to the habenular area of the brain and probably also to the pineal organ (because these fibers pass over the surface of the pineal organ on their way to the rest of the brain). The efferent fibers originate in the pineal organ and modify the parietal eye's response to light. The efferent nerves are not photosensitive, but they are chemosensitive; norepinephrine and serotonin, when applied to the pineal organ, will initiate activity in these nerves. Nerve cells within the pineal organ also give rise to nerve fibers that have been traced to the subependymal layer of the third ventricle. In addition, the pineal organ may itself be innervated by pinealopetal nerves which may be similar to the autonomic innervation observed in mammalian pineals (*7*).

Simultaneous surgical removal (*8*) of both the parietal eye and the pineal organ of Texas spiny lizards, *Sceloporus olivaceus*, free-running (expressing their endogenous circadian rhythmicity) in continuous illumination (LL) has one of three effects: (i) a "splitting" of the activity rhythm into two components, each of which "free-runs" with a different period (one case) (Fig. 1a); (ii) arrhythmicity (constant activity with no discernible period) (five cases) (Fig. 1b); or (iii) a marked change in the period of the free-running rhythm (six cases) (Fig. 1c). There was no apparent correlation between the intensity of LL and the effect observed. In six cases I sequentially removed the parietal eye and the pineal organ (Fig. 1d). Removal of the parietal eye alone had no effect on the free-running activity rhythm in any lizard, whereas subsequent removal of the pineal organ caused marked changes in the free-running period (three cases) (Fig. 1d) or arrhythmicity (three cases) (not shown in Fig. 1). Sham pinealectomies of several lizards (*8, 9*) had no effect on the free-running rhythms. The fact that effects are seen following pinealectomy but not parietalectomy is consistent with the hypothesis that the pineal organ alone is responsible for the observed effects. However, it would be premature to discard a possible involvement of the parietal eye, particularly in view of the intimate reciprocal innervation between these two organs. Unfortunately, it is not technically feasible to remove the pineal organ and leave the parietal eye unaffected, as pinealectomy would also sever the parietal eye nerve.

The pineal system does not represent the entire mechanism underlying circadian activity rhythms: (i) In many cases, rhythms persist in LL after pinealectomy, although with altered periods (Fig. 1, a, c, and d). (ii) Pinealectomized lizards are rhythmic in complete darkness (DD) (Fig. 1b). (Possible effects of pinealectomy on the period of the free-running rhythm in DD have not yet been tested.) (iii) Pinealectomy does not prevent entrainment (synchronization) of activity rhythms of lizards to 24-hour light-dark cycles (*9, 10*). The data are consistent with either of two hypotheses: (i) The pineal organ acts as a coupling device; some action of the pineal organ couples two or more circadian oscillators and maintains the fixed-phase relationships among them. (ii) The pineal organ acts as a master oscillator, which imposes its frequency on other circadian oscillators, to which it is coupled. The observation that pinealectomized lizards are rhythmic in DD (Fig. 1b) does not eliminate the possibility that the pineal organ may be the site of a master oscillator. This possibility can only be eliminated if pinealectomy is shown not to affect the *period* of the rhythm in DD.

The splitting observed after pinealectomy and parietalectomy (Fig. 1a) is, to my knowledge, and to date, the clearest demonstration in any vertebrate that the activity rhythm is controlled by at least two oscillators. There is not only an uncoupling between the two components of the activity rhythm but the two components (termed "short" and "long" oscillators) continue to free-run independently and crisscross several times. However, some mutual interaction persists between the two components, as reflected in small changes in the periods and amounts of activity of the two components when they cross. Also, from about 3 months after parietalectomy and pinealectomy, the motion of the short oscillator is no longer reflected in the lizard's overt activity. At this time there is a shortening of the long oscillator's period, which may indicate a rejoining of the two components. Splitting has never been seen in an intact lizard under any lighting condition (*9, 10*). Under certain experimental conditions, notably LL, the circadian activity rhythms of some other (intact) vertebrates (for example, starlings, hamsters, tupaias) also break into two components, and each component free-runs with a different frequency until they are approximately 180° out of phase with one another (*11*). At this phase relationship, they become coupled again and free-run with a similar period but 180° out of phase. These data have been interpreted as showing the existence of two oscillators controlling locomotor activity (*11*), but they may also be interpreted on the basis that a single oscillator with a complex waveform is driving the overt locomotor activity. In a few normal animals in LL (for example, hamsters and arctic ground squirrels), the split components do not become recoupled at 180° but scan one entire cycle (360°) before regaining a normal phase relationship (*10*). These few cases offer good evidence of the existence of two oscillators; however, the lizard activity shown in Fig. 1a is unique in that the two components scan several full cycles and thus serve as compelling evidence that at least two normally coupled circadian oscillators are responsible for the lizard's activity rhythm.

In birds and mammals, arrhythmicity in LL is sometimes preceded by splitting, which suggests that arrhythmicity may result from uncoupling of two or more circadian oscillators that drift out of phase with one another (*11*). The arrhythmicity that is seen after pinealectomy in lizards (Fig. 1b), therefore, is also consistent with the hypothesis that removal of the pineal organ has affected component oscillators of a multioscillator system by removing either a coupling device or a master oscillator.

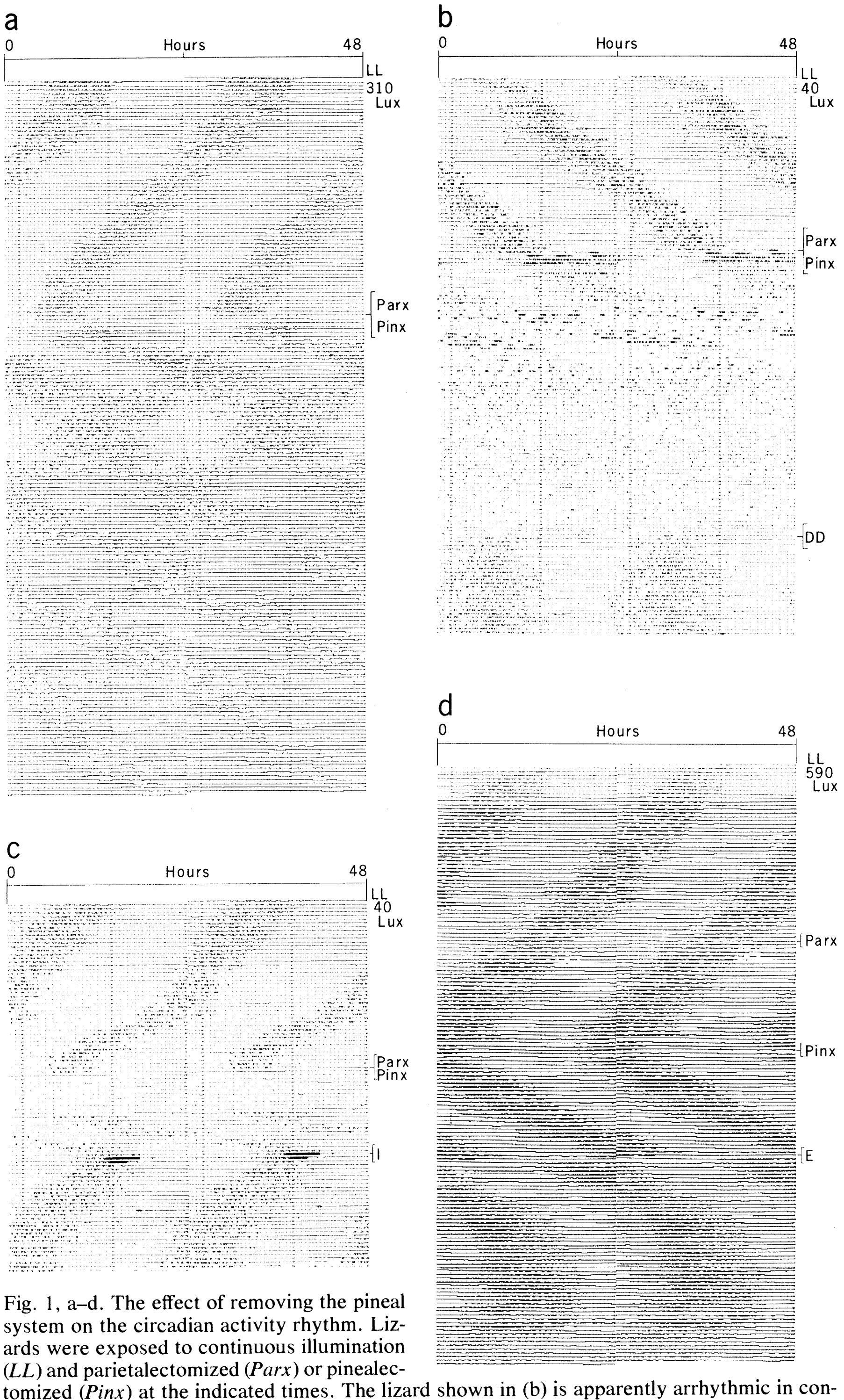

Fig. 1, a–d. The effect of removing the pineal system on the circadian activity rhythm. Lizards were exposed to continuous illumination (*LL*) and parietalectomized (*Parx*) or pinealectomized (*Pinx*) at the indicated times. The lizard shown in (b) is apparently arrhythmic in continuous illumination but is rhythmic in continuous darkness (*DD*). The heavy dark bars [*I* in (c)] are a recording artifact. At the point marked *E* in (d) the lizard escaped from its activity cage; it was returned to its cage in less than a day. To aid in interpretation the activity records are presented in duplicate, the right of half of each displaced 1 day above the left; each horizontal line, therefore, represents 48 hours of recording. Deflections of the baseline represent activity. Hour 0 is at midnight C.S.T.

Finally, the marked changes in the free-running period seen in some lizards after pinealectomy is also consistent with the hypothesis that pinealectomy affects coupling between two or more oscillators. In some cases (Fig. 1, c and d) pinealectomy produces first an abrupt lengthening in the free-running period and later a shortening. In these cases it seems likely that two oscillators are uncoupled by pinealectomy; one of these ("long oscillator") free-runs with a long period and drives the overt rhythm of activity, and the other ("short oscillator," usually not reflected by activity) free-runs with a period similar to that shown by the intact lizard (compare with Fig. 1a). When the two oscillators regain a normal phase relationship with each other, they again show some mutual entrainment; the long oscillator is at least temporarily influenced by the short oscillator and reflects the period of the short oscillator. The data suggest that, in most cases, the activity rhythm reflects the motion of only one of the uncoupled oscillators and that cases in which both oscillators are revealed by overt activity, such as the lizard with the split rhythm (Fig. 1a), are rare.

Among other vertebrate classes, the only areas that have been implicated in the control of circadian rhythmicity are the suprachiasmatic region of the hypothalamus of mammals and the pineal organ of birds. Lesions in the suprachiasmatic region of mammals abolish a variety of circadian rhythms (*1*). Pinealectomy in house sparrows abolishes the circadian rhythms of body temperature and perching activity; pinealectomized house sparrows are arrhythmic in DD (*2*). Inasmuch as interruption of the nervous input and output of the house sparrow's pineal organ does not abolish rhythmicity (*3*), the avian pineal seems to be hormonally, rather than neurally, coupled to other components of the circadian system. Furthermore, birds rendered arrhythmic by pinealectomy in DD can, in less than 24 hours, be made rhythmic by transplanting a pineal organ into the anterior chamber of the eye (*3*).

The pineal organs of both lizards and

birds seem to be crucial components of their timekeeping machinery, but major differences may exist between them (for example, pinealectomized birds are arrhythmic in DD whereas pinealectomized lizards are not). There is strong evidence that the avian pineal is the site of a master driving oscillator; the data presented here show that the lizard pineal is also an important component of circadian organization, but its exact function and the routes by which it is coupled to other components of the circadian system await elucidation.

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8. The parietal eye is easily removed; complete removal can be determined by inspecting under a dissecting microscope. The lizards were pinealectomized as described previously (*9*). Complete removal of the pineal organ was confirmed histologically in 14 of the 18 pinealectomized lizards. Four of the lizards died before the brains could be taken for histology. In sham pinealectomies, I drilled through the skull and exposed the brain but did not rupture the dura. The lizards were collected by noosing in the vicinity of Austin, Texas. The activity of individual lizards was monitored by "tilt cages" connected to an event recorder (Esterline Angus). The tilt cages were visually isolated from one another and exposed to constant fluorescent illumination of 40 or 310 lux (Westinghouse F96T12/cw bulbs) or 360 and 590 lux (General Electric F48T12/cw bulbs) in environmental chambers held at a constant 29°C. Food (live mealworms and tobacco hornworms) and water were freely available.
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Intravenous Self-Feeding: Long-Term Regulation of Energy Balance in Rats

Abstract. *Rats learned to press a lever for intravenous self-injection of liquid diet during periods of several weeks when oral food was not available. The intakes were low but regulated, and were sufficient to balance energy expenditures at low body weight. Systemic receptors alone are thus adequate to motivate feeding behavior and meter the caloric yield of the intravenous injections.*

The frequency of meals and their size together determine the daily food intake of animals. We do not fully understand the physiological states which generate these meal patterns, yet such information would considerably aid appetite control programs. When an animal begins to feed there is a decline of intracellular energy production (from all fuel sources) below a critical level (*1*, *2*). Sensory incentives, such as taste, have a modulating and sometimes overriding influence on these energy factors. The determinants of meal size are also complex, involving conditioned and unconditioned factors at peripheral (oral and gastric) as well as duodenal and systemic levels (*3*).

We have studied the capacity of systemic receptors alone to motivate and sustain ingestive behavior in rats (*4*). The participation of taste and other orogastric factors was eliminated by allowing the animals to feed themselves intravenously. We now report the characteristics of the long-term regulation of ingestion so achieved.

Adult male rats were fitted with permanently implanted intravenous (auricular) catheters. Our surgical preparation and infusion apparatus, described elsewhere (*5*), allows the animals complete freedom of movement. After a few days of postoperative recovery, the rats were deprived of oral food and given access to a lever which was positioned outside a window in the cage wall (in order to minimize accidental presses). Relays and timers caused each press on the lever to activate an infusion pump and deliver through the catheter a known amount of nutritive fluid from a 50-ml syringe. Several types of fluid were used, all of a concentration about 1.0 kcal/ml and containing most of the nutrient requirements of the rat (*2*, *6*). Water was freely available, but intakes were low despite the hypertonicity of the self-injected fluids.

The present results were obtained from 30 rats that did not develop pathological or other problems such as leaks in the infusion line. These rats were studied for 5 to 30 consecutive days. Although they had had no prior experience with levers, some 70 percent of them self-administered a fairly constant amount of nutritive fluid from day 2 or day 3 of the experiment. The remaining 30 percent of the rats showed little spontaneous pressing, and had to be attracted to the bar by placing a few drops of sweet fluid on it. Operant responding was immediately initiated, and no further oral incentives were given. Thereafter, all of the rats deliberately activated the infusion pump with a single press of the lever. Most rats were placid or groomed a little during injections, but three animals consistently chewed the lever. After steady responding was established, the daily intakes were 27 kcal per 24 hours (range, 15 to 50 kcal); no arbitrary "learning criterion" was applied. Presses of the lever were distributed throughout the 24 hours, with the highest density occurring around nightfall (Fig. 1). This corresponds to the time of maximal locomotor activity, hence of energy expenditure. In terms of calories, the amount received by each rat per injection (quantum) was most usually

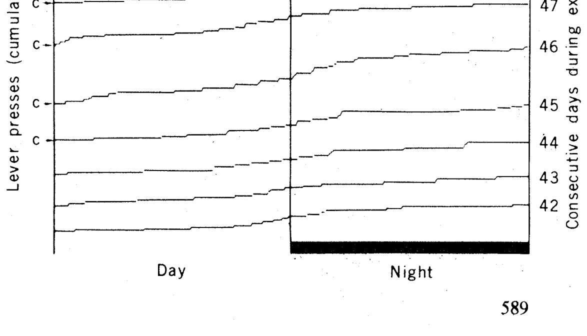

Fig. 1. Cumulative record (expressed as lever presses) of intravenous self-feeding in rat 178 on consecutive days. The concentration (*c*) of the infused fluid was halved on days 45 to 48, with an average increase of 40 percent in the volume injected.