J Comp Physiol (1981) 144:381–389

Journal
of Comparative
Physiology · A

Pineal and Hypothalamic Pacemakers: Their Role in Regulating Circadian Rhythmicity in Japanese Quail

S.M. Simpson and B.K. Follett
ARC Research Group on Photoperiodism and Reproduction, Department of Zoology, The University, Bristol BS8 1UG England

Accepted July 7, 1981

Summary. Neither pinealectomy nor administration of melatonin *via* silastic capsules had any effect on free-running circadian rhythms of locomotor activity in Japanese quail (*Coturnix coturnix japonica*). The quail, like the chicken, therefore differs from sparrows and starlings in which pinealectomy dramatically disrupts free-running rhythms. Nevertheless, it seems unlikely that there are fundamental differences in circadian organisation within the Class Aves. The effects of lesions within the supraoptic region (SOR) of the hypothalamus were similar to those which follow the ablation of the suprachiasmatic nuclei (SCN) in sparrows, rats and hamsters, causing the breakdown of free-running rhythms of locomotor activity, but not necessarily an arrhythmic state. The SOR and SCN appear then to have homologous functions in birds and mammals. Differences in circadian organisation, such as the degree of influence of the pineal gland and the particular photoreceptors used for entrainment, may therefore be modifications peripheral to the fundamental components of the circadian clock.

Introduction

The pineal gland is thought to play a central role in the circadian organisation of several avian species. In the house sparrow (*Passer domesticus*) it is required for the normal expression of the free-running rhythms of locomotor activity and of body temperature, and pinealectomy causes arrhythmia in constant conditions (Gaston and Menaker 1968; Binkley et al. 1971; Zimmerman and Menaker 1975; Menaker and Zimmerman 1976). The transplantation of a pineal gland to a previously arrhythmic pinealectomized sparrow not only restores rhythmicity but also confers upon the recipient the circadian phase of the donor (Zimmerman and Menaker 1979). Rhythmicity of activity has also been abolished by pinealectomy in three other species of sparrow (Gaston 1971; McMillan 1972; Ebihara and Kawamura 1980). Likewise, the gland appears important for normal rhythmicity in starlings (*Sturnus vulgaris*) although here, pinealectomy does not necessarily lead to arrhythmia (Rutledge and Angle 1977) but often results in a shortening of the free-run period (τ), a lengthening in the duration of activity in each circadian cycle (α), and instability in both of these parameters (Gwinner 1978). These results, together with the observation that pinealectomized sparrows and starlings are still able to entrain to light/dark cycles, has suggested that the circadian rhythm of locomotor activity is controlled by one or more subordinate oscillators which are normally coordinated by a humoral output from the pineal gland, possibly melatonin (Menaker and Zimmerman 1976; Gwinner 1978). This interpretation is supported by the results of administering melatonin to house sparrows in constant darkness (DD), a treatment which causes either a dramatic shortening in τ, or continuous, virtually arrhythmic activity (Turek et al. 1976). Furthermore, the free-running activity rhythm of pinealectomized starlings can be entrained by daily injections of melatonin, although it remains possible that this effect could be indirect since melatonin has effects on the sleep-wakefulness cycle (Gwinner and Benzinger 1978).

The extent to which the scheme outlined above applies to other vertebrates is still far from clear. In two species of lizard pinealectomy upsets the circadian activity rhythm, causing either its splitting into two components or marked changes in τ or arrhythmicity, whilst melatonin administration has effects similar to those seen in sparrows (Underwood 1977, 1979). However, in golden hamsters and rats the pine-

---

Abbreviations: POR preoptic area; *SOR* supraoptic region; *SCN* suprachiasmatic nuclei

0340-7594/81/0144/0381/$01.80

al gland has little if any effect on overt circadian rhythms (Quay 1968, 1970a, b; Finkelstein et al. 1978). Since it also appears that pinealectomy may have no effects upon the free-running activity rhythms of chickens (MacBride 1973; cited in Menaker and Zimmerman 1976), the extent to which the pineal gland is involved in circadian organisation even within the Class Aves, is far from a closed topic, and in this paper we discuss its possible role in the Japanese quail (*Coturnix coturnix japonica*). A related issue concerns the role of the suprachiasmatic nuclei (SCN), located in the anterior hypothalamus, in regulating circadian rhythmicity. In hamsters and rats their destruction abolishes a host of endocrine and metabolic circadian rhythms as well as oestrous cyclicity and the photoperiodic response (Moore and Eichler 1972; Stephan and Zucker 1972; Rusak and Morin 1976; Stetson and Watson-Whitmyre 1976; Brown-Grant and Raisman 1977; Rusak 1977; for review see Rusak and Zucker 1979). Inevitably, this has raised the question as to whether the SCN are another possible central pacemaker in birds and recently it has been reported that lesions of the SCN in both house sparrows and Java sparrows abolish free-running activity rhythms (Takahashi and Menaker 1979a; Ebihara and Kawamura 1980). Comparable experiments in quail are described in this paper.

An important reason for our interest in the central neural control of circadian rhythmicity in birds arises from a long-standing research programme into the photoperiodic control of reproduction in birds, using the quail as a model species. Daylength measurement in both birds and mammals involves a clock that has a circadian component (reviews, Follett 1973; Follett et al. 1981) but nothing is known of its anatomical basis. However, it is known that three areas of the quail's hypothalamus – the ventral and posterior infundibular nuclear complexes, and the preoptic area – are essential for the photoperiodic induction of gonadotrophin release (Sharp and Follett 1969; Stetson 1972a, b; Oliver and Baylé 1973; Davies and Follett 1975a, b; Davies 1980). The precise functions of these areas remain unclear, but one or more could well be involved in the extra-retinal perception of light that is essential for the avian photoperiodic response (Benoit 1970; Oliver and Baylé 1976; Yokoyama et al. 1978; Homma et al. 1979). Alternatively, these areas might elaborate the Gn-RH neurosecretions which regulate LH and FSH secretion from the pituitary gland, or contain various neural interconnections, or be in some way associated with the circadian clocks underlying photoperiodic time-measurement. To locate such circadian clocks would be an important step in understanding the hypothalamic control of seasonal reproduction and so, prompted by the importance of the SCN in rodents and sparrows, we have investigated the role played by the anterior hypothalamus in the photoperiodic response and in the circadian rhythms of locomotor activity. Preliminary findings have been reported previously (Simpson and Follett 1980) and have also been discussed in recent reviews (Takahashi and Menaker 1979a, b; Menaker et al. 1981). The formal properties of the circadian rhythm of locomotor activity in the quail are discussed in Simpson and Follett (1981a).

Materials and Methods

Maintenance of Birds and Activity Recording. Japanese quail were raised from hatch under a photoperiod of LD 8:16 until they were 5–6 weeks of age. The sexually immature males were then placed singly in tilt-floor activity recording cages (16 × 21 × 26 cm), each contained in a light-tight-box (51 × 36 × 31 cm). All boxes were fitted with an 8 W fluorescent light (Warmwite, Philips Ltd) producing a light intensity of about 700 lux on the cage floor, and a loudspeaker emitting a low level of white noise. Turkey starter crumbs and water, available *ad libitum*, were replenished every 16–18 days at which time the birds were exposed briefly to white light of about 50 lux.

Pinealectomy. Quail were maintained in their activity recording boxes in darkness (DD) for between 2 and 12 weeks before pinealectomy or control surgery, and for at least 5 weeks afterwards. Before operation each quail was anaesthetized by first giving two 0.2 ml doses of a solution containing 35% v/v propylene glycol, 9.5% v/v ethanol, 4.2% w/v chloral hydrate, 1% w/v sodium pentobarbitone and 0.2% w/v magnesium sulphate. This was followed by a 0.2 ml dose of 1% w/v Hypnodil (Janssen Ltd). The injections were given intramuscularly at 5 min intervals. The pineal gland, and usually the attached choroid plexus, were removed with mouse-tooth forceps through a small hole cut in the roof of the skull. Control operations were identical except for the removal of the pineal gland and choroid plexus. At the end of the experiment the birds were killed, and their heads fixed in Bouin's fluid. After being treated for several hours with a rapid-decalcification solution (RDC, Lamb Ltd) the tissue, which included the cranium over the site of the pineal gland, was prepared for histology (15–20 μM sagittal or coronal sections, stained with Ehrlich's haematoxylin and eosin, or Mallory's triple stain). The presence of any pineal tissue was then carefully assessed microscopically.

Implantation of Melatonin-Filled Capsules. Capsules of three different lengths were prepared from Silastic tubing (Dow Corning, external diameter 2.41 mm, internal diameter 1.57 mm) and filled with melatonin (Sigma Ltd) before being sealed with Dow Corning elastomer. Capsules were implanted subcutaneously in the neck region of quail that had been held in darkness for at least 4 weeks prior to the operation. Six quail were implanted with 2 mm capsules, 6 with 20 mm capsules and 4 with 40 mm capsules. A further group of 7 quail were treated with empty capsules as controls.

Electrolytic Lesions of the Anterior Hypothalamus. After a 2–3 week period in darkness quail were anaesthetized and placed in a sterotaxic frame. An opening was made in the roof of the skull with a dental burr and an electrode (made from an insect pin of 0.15 mm diameter and insulated to within 0.2 mm of the tip with Bakelite) lowered to predetermined coordinates 0.3 mm lateral to the midline. The exact position of the electrode tip was located *in situ* by X-ray photography and any necessary adjustments made. A lesion was made by passing a 3 mA DC current for 10 s (apparatus of Fleming 1957). The electrode was then withdrawn and replaced at the same coordinates 0.3 mm on the other side of the mid-line and a second lesion made. After recovery the birds were returned to darkness for a further 5 to 10 weeks, after which they were exposed to LD 20:4 for 2–3 weeks to check for photoperiodically induced testicular growth. They were then killed, the testes weighed and the brains processed histologically (Bouin's fixation, 15 µm sagittal sections stained with Mallory's triple stain). For more detail of the lesioning techniques and the stereotaxic atlases used see Baylé et al. (1974), Davies and Follet (1975a, b), Davies (1980).

Analysis of Activity Records. Daily records of each bird's locomotor activity were produced on Esterline Angus chart paper and after mounting in the standard fashion the overall records were photographically double-plotted to enhance the interpretation of rhythmic features.

Free-run periods were determined by regression analysis from the times of either 6 consecutive activity onsets, or 6 ends of activity (Daan and Pittendrigh 1976). Average curves were calculated for the pre- and postoperative data of the pinealectomized and control birds. This was done by scoring the data for each hour of 4 successive circadian cycles 2 weeks before operation, and from 4 circadian cycles afterwards. A single pen deflection was counted as one point, whilst a solid block of continuous activity lasting 1 mm was assigned 12 points. For each bird the activity score in each circadian hour ($\tau/24$) was averaged over the 4 cycles and reduced to a percentage of the total activity. Using the hour during which activity ended as time zero for each bird, the average for each hour from the birds in a particular experimental group were combined to form an overall average curve. The 4 average curves so formed were then subjected to an analysis of variance for repeated measures (Winer 1971).

Results

Effects of Pinealectomy on the Free-Running Activity Rhythm

In all 21 birds which displayed good free-running activity rhythms in DD were pinealectomized. Later histological examination revealed that 14 of these had minute pieces of putative pineal gland tissue remaining and so they were excluded from further analysis. Seven birds were considered to have been completely pinealectomized, although some minor damage to the cerebellum and cerebral hemispheres immediately adjacent to the site of the pineal gland and the presence of parenchymatous cells made it impossible to exclude the possibility that a few pineal cells remained. The activity records of one pinealectomized and one control-operated bird are shown in Fig. 1.

Subjective analysis of all the records showed that neither partial nor complete pinealectomy, nor the control operations, caused loss of normal free-running rhythmicity in DD. No instances of arrhythmia following pinealectomy were observed and, with the possible exception of one bird, there were no obvious changes in the duration of activity (α) in each circadian cycle. Activity records for the 2 week period before operation and for between 2 and 5 weeks afterwards were analysed quantitatively. The mean ($\pm$ S.E.M.) free-run period (τ) of the control birds ($n=7$)

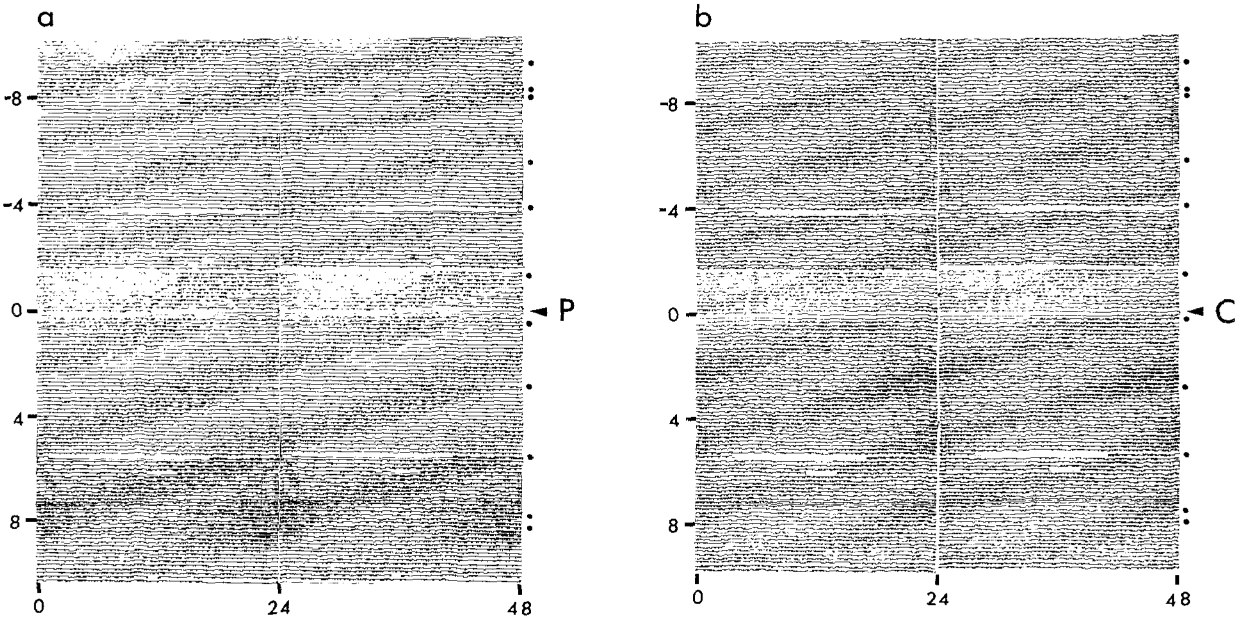

Fig. 1. **a** Double-plotted activity recording of a quail that was pinealectomized (*P*) on the day indicated. **b** Activity recording of a quail that was control operated (*C*) on the day indicated. Food and water were replenished or the bird otherwise disturbed on the days indicated (●). Numbers on the horizontal axis indicate the time in hours and those on the vertical axis the number of weeks before and after operation

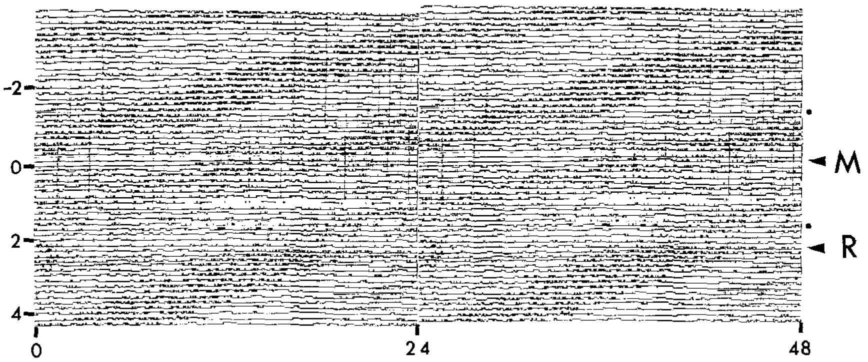

Fig. 2. Activity recording of a quail in constant darkness. A 20 mm melatonin-filled capsule was implanted subcutaneously (*M*) and removed (*R*) on the days indicated. For further explanation of symbols see Fig. 1

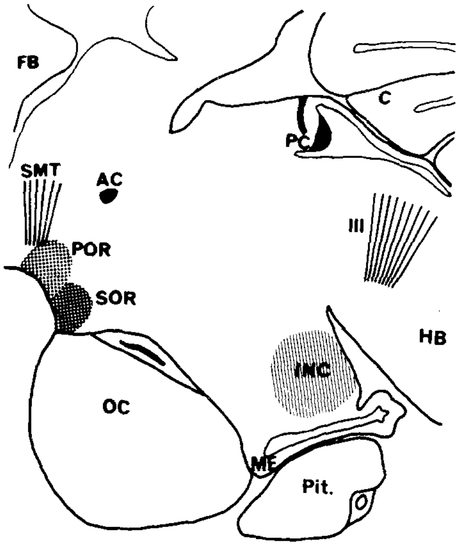

Fig. 3. Diagrammatic representation of a mid-sagittal section of a quail's hypothalamus. *AC* anterior commissure; *C* cerebellum; *FB* forebrain; *INC* infundibular nuclear complex; *HB* hindbrain; *ME* median eminence; *OC* optic chiasma; *PC* posterior commissure; *Pit* pars distalis of the pituitary gland; *SMT* septomesencephalic tract; *III* oculomotor nerve. From Davies (1980)

was 23.0 ± 0.2 h before operation and 22.6 ± 0.2 h afterwards. In pinealectomized birds ($n = 7$) the mean pre- and postoperative free-run periods were, respectively, 22.8 ± 0.1 and 22.6 ± 0.2 h. An analysis of variance for repeated measures showed that there was no significant difference between τ of control and pinealectomized birds either before, or after surgery ($P > 0.05$). Also, the period τ did not change significantly following either type of operation ($P > 0.05$). The unsigned difference between the pre- and postoperative free-run period of each bird was also calculated to indicate whether or not there was a difference between pinealectomized and control birds in any tendency to τ to change, irrespective of direction, after operation. No significant difference between the change in τ of control and pinealectomized birds was found (Student's t-test, $P > 0.05$). Average curves were constructed to describe the pattern of activity and rest during the circadian cycle. No significant differences were found between the average curves of the control and pinealectomized birds (analysis of variance for repeated measures, $P > 0.05$).

Implantation of Melatonin-Filled Capsules in Free-Running Quail

Free-run periods were determined from either the onsets or the ends of activity for 6 consecutive circadian cycles up to 2 weeks before and 5 weeks after implantation, and 2 to 4 weeks following capsule removal. No significant changes in τ occurred either after implantation with melatonin (where free-running rhythms could be discerned) or after their removal (analysis of variance for repeated measures, $P > 0.05$). Thus the mean free-run periods before, during and after implantation of a 2 mm capsule ($n = 7$) were, respectively, 22.4 ± 0.2, 22.6 ± 0.2 and 22.8 ± 0.1 h, and in birds treated with 20 mm capsules ($n = 4$), 22.6 ± 0.3, 23.2 ± 0.6 and 22.7 ± 0.3 h.

While empty capsules had no effect whatsoever on the pattern of activity, the peak of locomotor activity marking the end of the active phase was reduced in intensity in 3 of the 6 quail implanted with 2 mm melatonin-filled capsules, and in 3 of 4 birds with 20 mm capsules which were still clearly rhythmic, the overall level of activity was reduced. A typical example is shown in Fig. 2. In the remaining 4 birds with 20 mm capsules and the 4 birds with 40 mm implants, the locomotor activity during the active phase was reduced and that during the rest phase increased to such an extent that periodicity in the data was obscured and activity became virtually arrhythmic. In these birds the mean free-run period before implantation was not significantly different from that after capsule removal (Student's t-test, $P > 0.05$) and were, respectively, 22.9 ± 0.2 and 23.0 ± 0.4 h in birds treated with 20 mm capsules, and 22.8 ± 0.4 and 23.3 ± 0.3 h in those treated with 40 mm capsules.

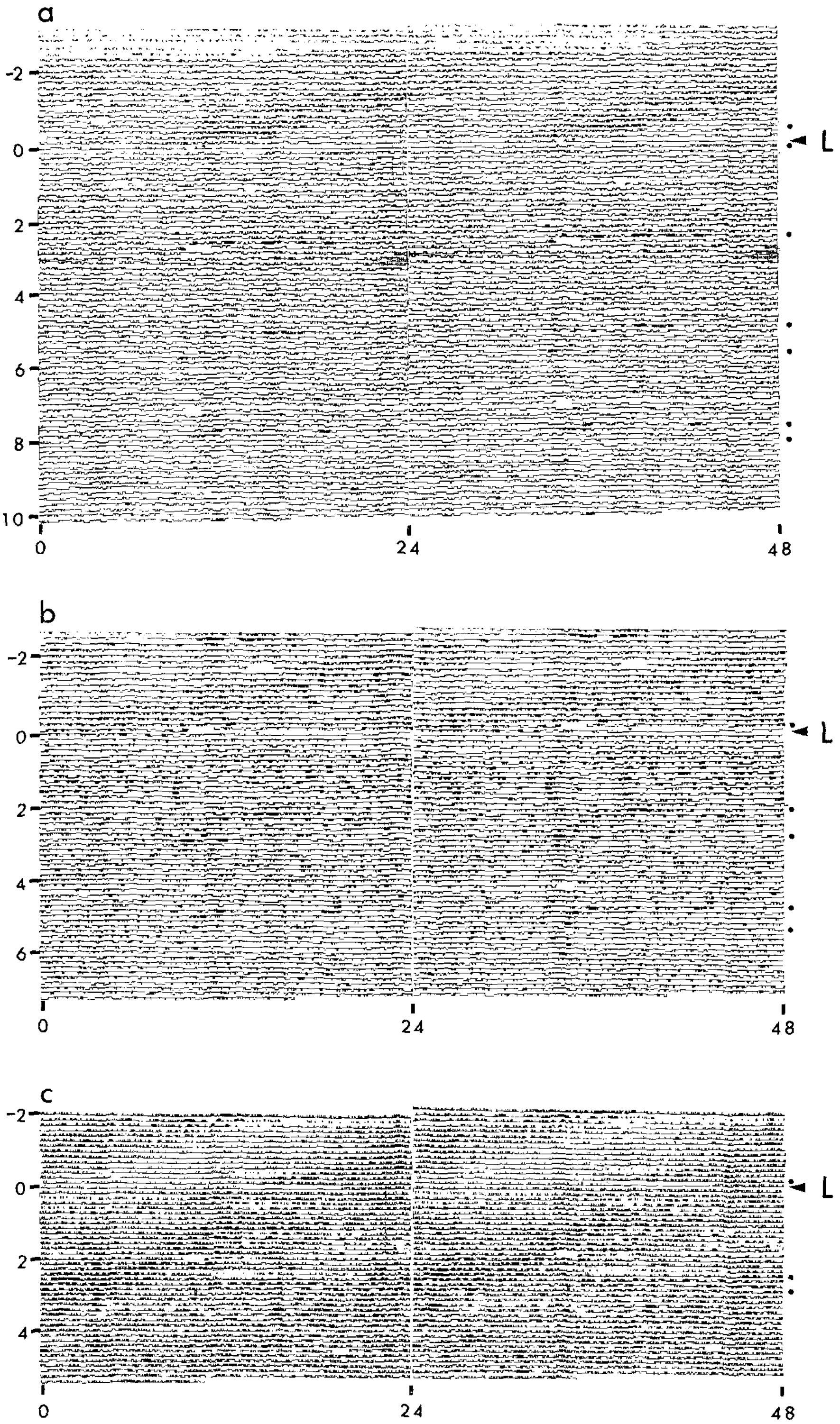

Fig. 4a–c. Activity recordings of three quail in constant darkness. Lesions of the SOR were made on the day indicated (*L*) which resulted in either complete loss of the circadian pattern of activity (**a**, **b**) or a progressive loss of clarity of the circadian pattern (**c**). For further explanation of symbols see Fig. 1

Electrolytic Lesions of the Anterior Hypothalamus

Histology was completed on 27 birds and showed that the bilateral lesions merged in the midline, and were close to, often penetrating, the ventral surface of the brain anterior to the optic chiasma. The lesioned area extended up to 500 μm in the sagittal plane and up to 500 μm either side of the midline.

Five quail had lesions within the preoptic area (POR), that is, the region below the line of descent of the septomesencephalic tracts (Fig. 3). Their activity rhythms were normal. The other 22 birds had slightly more posterior lesions. The area of destruction included at least part of the POR but also included the supraoptic region (SOR), the region immediately anterior to and abutting the rostral end of the optic chiasma (Fig. 3). The normal circadian rhythm of locomotor activity of 10 of these birds was disrupted by the lesion (Fig. 4a–c). While circadian rhythmicity continued for at least 2 weeks after operation in these birds, the normally clear distinction between the activity and rest phases was reduced in 3 birds to the extent that rhythmicity was almost completely obscured. Figure 4a, for example, shows an activity pattern which was virtually arrhythmic for 2 weeks after operation, although during the third week a distinction can be made between an active phase and a rest phase. Subsequently, there was a further loss of the clarity of the circadian rhythms of all 10 birds. The 3 birds initially most affected by the lesion apparently became arrhythmic until the end of the experiment. However, close examination of the records reveals that each shows several groups of activity bursts which recur at intervals within the circadian range and thus appear to free-run independently of one another. Examples of these can be seen in Fig. 4a between weeks 3 and 5, and in Fig. 4b between weeks 3 and 5, and between week 6 and the end of the record. While these could be due to chance alignment of otherwise random distributions of activity bursts, they could also represent the presence of several weakly rhythmic components. That rhythmicity was not totally abolished in these birds is supported by the 're-emergence' of an indistinct circadian pattern between weeks 8 and 10 in one bird (Fig. 4a). In the other 7 birds affected by the lesion, there was a progressive reduction in the clarity of the circadian rhythms, although never to the same extent as those which appeared to become arrhythmic (e.g. Fig. 4c).

None of the birds in the study was photoperiodically blocked: the cloacal glands, which are androgen-dependent organs, were undeveloped in all birds after prolonged exposure to constant darkness, indicating that there had been no testicular development, while after photostimulation the mean testicular weight was 1431 ± 116 mg ($n=27$), a value not different from that in intact quail exposed to maximally stimulatory daylengths (see Follett and Maung 1978).

Discussion

As emphasised in the Introduction, pinealectomy in four species of sparrow, as well as the starling, disrupts the free-running circadian rhythms of locomotor activity in constant conditions. In these species, therefore, the pineal gland appears to play an important and integral part in the circadian system, maintaining mutual phase relationships between subordinate oscillators and, together, generating the circadian rhythm of activity (review, Menaker et al. 1981). In contrast, removal of the chicken's pineal gland does not seem to have an adverse effect on free-running activity (MacBride 1973) and in this study on the Japanese quail, another gallinaceous bird, no significant changes in τ or the activity pattern followed pinealectomy. Here, then, are two species in which the pineal gland does not appear crucial for the normal generation of the circadian rhythm of locomotor activity.

The results of administering melatonin add weight to the view of real differences between bird species. In house sparrows melatonin given in Silastic capsules leads to considerable changes in τ (Turek et al. 1976), suggesting that melatonin may indeed be the humoral agent from the pineal gland which coordinates the subordinate oscillators within the brain. Treatment of free-running quail with melatonin caused a dose-related reduction in the intensity of locomotor activity, consistent with its previously reported sedative effect on birds (Hishikawa et al. 1969; Binkley 1974; Hendel and Turek 1978) but no effects on the circadian system were apparent. This insensitivity, even to pharmacological doses, rather suggests that neither pineal melatonin nor melatonin from any other site (e.g. the eye) are important in regulating the circadian features of the quail's activity rhythm.

What explanations can be offered for the variation between species in their apparent dependence upon the pineal as a pacemaker? It seems unlikely that the distinctions are due to fundamental differences in the circadian systems of birds, particularly since the chicken's pineal gland appears to contain a circadian clock that regulates melatonin secretion both in vivo and in vitro (review, Menaker et al. 1981). The choice of experimental conditions has been suggested as one possible source of variation (Takahashi and Menaker 1979a, b), but it also seems reasonable to assert that a series of modifications to some basic organisation could produce marked inter-species variation. Evolution could have operated on the several components that go to make up the circadian system in such a way that the relative importance of the components alters from species to species. Thus, the several hypothetical oscillators regulating activity rhythms in the sparrow appear to be weakly coupled with the result that pineal removal leads to arrhythmia, whereas in the starling the coupling is somewhat stronger and the consequent effects of pineal removal less dramatic (Menaker and Zimmerman 1976; Gwinner 1978). If chickens and quail do indeed share the same basic circadian system the couplings must be even more robust since after pineal removal both free-running and, at least in the quail, entrainment (unpublished observations) appear to be unaffected. If marginal effects of pinealectomy could be demonstrated in quail or chickens, this would greatly strengthen the argument that avian circadian systems are built around a common core of components but with a variety of internal coupling strengths and degrees of dependence upon the pineal gland as an integrator. Perhaps there are adaptive advantages in the particular entrainment properties conferrred by each strategy.

The strongest 'positive' result in this study arises from the effects of lesions within the supraoptic region (SOR), an area that includes the suprachiasmatic region (distinct SCN are not found in quail). Rhythmicity in such lesioned quail usually 'decayed' over several weeks producing activity patterns in which circadian rhythmicity was either barely discernable, or reduced to a few very weak periodicities. The continuation of at least weak rhythmicity in most birds might indicate that it was not the circadian system which was disrupted by the lesion, but rather a centre, normally controlled by the circadian system, which regulates the state of wakefulness and rest. Alternatively, these results might be due to an effect on the circadian system itself, perhaps as a result of the incomplete ablation of a 'master oscillator', or because there are subordinate systems elsewhere that are capable of expressing some degree of rhythmicity. Support for some type of rhythmicity persisting after SCN lesions is available in both birds and mammals. While there is loss of rhythmicity in SCN-lesioned house sparrows held in constant conditions, inspection of the published record (Takahashi and Menaker 1979a) reveals that, as in the quail, loss of rhythmicity does not seem to occur immediately after lesioning but gradually over several days, and there is some indication of a re-emergence of rhythmicity towards the end of the record. In addition, entrainment of SCN-lesioned house sparrows is still possible. Persistent rhythmicity following SCN removal is even better documented for the golden hamster (Rusak 1977, 1979). Wheel running is not necessarily totally arrhythmic and periodicities may remain which in darkness (DD) can temporarily couple together or free-run separately, and which can also be entrained by light cycles. These close similarities in the effects of SCN lesions suggest that the area may have homologous functions in rodents, sparrows and quail. This conclusion would be better founded if, following SCN ablation in birds, circadian functions other than activity were disrupted as in rodents. In the present study lesions which disrupted free-running locomotor activity were ineffective in preventing the photoperiodic control of gonadal growth, a response which we believe involves the use of circadian oscillators to measure daylength. One possible explanation for these results is that only part of the circadian system had been destroyed by the lesion, and whilst abolishing the ability to free-run in constant darkness, did not prevent entrainment. Subordinate oscillatory units or remnants of the master oscillatory system might then have entrained to the LD 20:4 cycle with sufficient 'internal' or 'external' coincidence to induce gonadal development (reviews, Pittendrigh 1972, 1981; Follett et al. 1981). Such interpretations are certainly testable in future experiments, for example, by carefully examining the photoperiodic threshold of such lesioned birds or by exposing them to cycles designed to indicate whether photoperiod is being measured by the circadian system.

Other explanations for the apparent failure to affect photoperiodic time-measurement centre around the question as to whether the SOR is involved in the particular circadian system measuring daylength. There is evidence which, when interpreted in terms of the external coincidence hypothesis, suggests that the circadian systems underlying locomotor activity and the photoperiodic response in quail are not one and the same, and show substantial quantitative differences in their entrainment to light/dark cycles (Simpson and Follett 1981b). Just possibly the two circadian systems are localised separately within the brain. The issue is confounded by Davies' discoveries of the effects of lesions within the POR/SOR on reproductive function in quail (Davies and Follett 1975a, b; Davies 1980). Lesions in the POR block photoinduced gonadal growth whilst lesions in the SOR disrupt ovulation but not ovarian growth on long daylengths. Circadian rhythms are involved in both processes: The time of ovulation (Konishi 1980) and photoperiodic time-measurement. It is tempting, therefore, to ascribe some of these lesion effects to disruption of the circadian systems but this may be a naive view of the manifold functions carried out by the anterior hypothalamus, and disruption of reproductive function would certainly develop, for example if the Gn-RH cell bodies in this region were destroyed. If the blockage to ovulation was brought about by the disruption of the circadian system regulating the pre-ovulatory surge in LH, then the SOR in the quail would appear to be involved in at least two circadian-based functions, ovulation and locomotor activity, and might therefore be a central part of a circadian system which is either analogous to, or homologous with, that of rodents. There are, however, indications of considerable divergence between avian and mammalian circadian systems. These are found in the different importance each attaches to the eyes and extra-retinal photoreceptors in entrainment and photoperiodic responses, and in the continuation of circadian rhythmicity in the in vitro avian pineal gland, a feature not shared by the denervated pineal gland (review, Rusak and Zucker 1979). The failure to affect overt rhythmicity in golden hamsters and rats by pinealectomy could be taken as further evidence of their having a very different circadian organisation from that of birds, but these are essentially negative results like those which follow the pinealectomy of chickens and quail. The pineal gland plays an as yet ill-understood part in the photoperiodic responses of golden hamsters, Sibirian hamsters, voles and ferrets, where it may function either as an effector of the circadian system or as an integral part of it (reviews, Hoffmann 1981; Herbert 1981). The rhythmic properties of the pineal gland may then have been utilized, albeit for different specific functions, by what may be fundamentally the same circadian system in birds and mammals. One implication of this interpretation is that the differences in the effects of pinealectomy which are found between bird species and between birds and mammals, like the differences in the particular photo-receptors used by each group, might be due to modifications peripheral to the central circadian clock.

References

Baylé J-D, Ramade F, Oliver J (1974) Stereotaxis topography of the brain of the quail (*Coturnix coturnix japonica*). J Physiol (Paris) 68:219–241

Benoit J (1970) Étude de l'action des radiations visibles sur la gonadostimulation et de leur pénétration intracranienne chez les oiseaux et les mammifères. In: Benoit J, Assenmacher I (eds) La photorégulation de la reproduction chez les oiseaux et les mammifères. CNRS, Paris, pp 121–146

Binkley S (1974) Pineal and melatonin: Circadian rhythms and body temperature of sparrows. In: Sheving LE, Halberg F, Pauly JE (eds) Chronobiology. Shoin, Tokyo, pp 582–585

Binkley S, Kluth E, Menaker M (1971) Pineal function in sparrows: Circadian rhythms and body temperature. Science 174:311–314

Brown-Grant K, Raisman G (1977) Abnormalities in reproductive function associated with the destruction of the suprachiasmatic nuclei in female rats. Proc R Soc Lond [Biol] 198:279–296

Daan S, Pittendrigh CS (1976) A functional analysis of circadian pacemakers in nocturnal rodents. II. The variability of phase response curves. J Comp Physiol 106:253–266

Davies DT (1980) The neuroendocrine control of gonadotrophin release in the Japanese quail III. The role of the tuberal and anterior hypothalamus in the control of ovarian development and ovulation. Proc R Soc Lond [Biol] 206:421–437

Davies DT, Follett BK (1975a) The neuroendocrine control of gonadotrophin release in the Japanese quail. I. The role of the tuberal hypothalamus. Proc R Soc Lond [Biol] 191:285–301

Davies DT, Follett BK (1975b) The neuroendocrine control of gonadotrophin release in the Japanese quail. II. The role of the anterior hypothalamus. Proc R Soc Lond [Biol] 191:303–315

Ebihara S, Kawamura H (1980) Central mechanism of circadian rhythms in birds. In: Tanabe Y, Tanaka K, Ookawa T (eds) Biological rhythms in birds. Neural and endocrine aspects. Springer, Berlin Heidelberg New York, pp 71–78

Finkelstein JS, Baum FR, Campbell CS (1978) Entrainment of the female hamster to reversed photoperiod: Role of the pineal. Physiol Behav 21:105–111

Fleming DG (1957) A constant current apparatus for the production of electrolytic lesions. Electroencephalogr Clin Neurophysiol 9:551–554

Follett BK (1973) Circadian rhythms and photoperiodic time measurement in birds. J Reprod Fertil Suppl 19:5–18

Follett BK, Maung SL (1978) Rate of testicular maturation, in relation to gonadotrophin and testosterone levels, in quail exposed to various artificial photoperiods and to natural daylengths. J Endocrinol 78:267–280

Follett BK, Robinson JE, Simpson SM, Harlow CR (1981) Photoperiodic time measurement and gonadotrophin secretion in quail. In: Follett BK, Follett DE (eds) Biological clocks in seasonal reproductive cycles. Wright, Bristol, pp 185–197

Gaston S (1971) The influence of the pineal organ on the circadian activity rhythms in birds. In: Menaker M (ed) Biochronometry. National Academy of Sciences, Washington, DC, pp 541–549

Gaston S, Menaker M (1968) Pineal function: The biological clock in the sparrow? Science 160:1125–1127

Gwinner E (1978) Effects of pinealectomy on circadian locomotor activity rhythms in European starlings *Sturnus vulgaris*. J Comp Physiol 126:123–129

Gwinner E, Benzinger I (1978) Synchronisation of a circadian rhythm in pinealectomized European starlings by daily injections of melatonin. J Comp Physiol 127:209–213

Hendel RC, Turek FW (1978) Suppression of locomotor activity in sparrows by treatment with melatonin. Physiol Behav 21:275–278

Herbert J (1981) The pineal gland and the photoperiodic control of the ferret's reproductive cycle. In: Follett BK, Follett DE (eds) Biological clocks in seasonal reproductive cycles. Wright, Bristol, pp 261–275

Hishikawa Y, Cramer H, Kuhlo W (1969) Natural and melatonin-induced sleep in young chickens – a behavioural and electrographic study. Exp Brain Res 7:84–94

Hoffmann K (1981) The role of the pineal gland in the photoperiodic control of seasonal cycles in hamsters. In: Follett BK, Follett DE (eds) Biological clocks in seasonal reproductive cycles. Wright, Bristol, pp 237–249

Homma K, Ohta M, Sakakibara Y (1979) Photoinducible phase of the Japanese quail detected by direct stimulation of the brain. In: Suda M, Hayaishi O, Nakagawa H (eds) Biological rhythms and their central mechanism. Elsevier/North Holland, Amsterdam, pp 85–94

Konishi T (1980) Circadian rhythm of ovipositional time in Japanese quail. In: Tanabe Y, Tanaka K, Ookawa T (eds) Biological rhythms in birds. Neural and endocrine aspects. Springer, Berlin Heidelberg New York, pp 79–90

MacBride SE (1973) Pineal biochemical rhythms of the chicken (*Gallus domesticus*): light cycle and locomotor activity correlates. PhD thesis, University of Pittsburgh

McMillan J (1972) Pinealectomy abolishes the circadian rhythm of migratory restlessness. J Comp Physiol 79:105–112

Menaker M, Zimmerman N (1976) Role of the pineal in the circadian system of birds. Am Zool 16:45–55

Menaker M, Hudson DJ, Takahashi J (1981) Neural and endocrine components of circadian clocks in birds. In: Follett BK, Follett DE (eds) Biological clocks in seasonal reproductive cycles. Wright, Bristol, pp 171–183

Moore RY, Eichler VB (1972) Loss of a circadian adrenal corticosterone rhythm following suprachiasmatic lesions in the rat. Brain Res 42:201–206

Oliver J, Baylé J-D (1973) Photically evoked potentials in the gonadotropic areas of the quail hypothalamus. Brain Res 64:103–121

Oliver J, Baylé J-D (1976) The involvement of the preoptic-suprachiasmatic region in the photosexual reflex in quail: Effects of selective lesions and photic stimulation. J Physiol (Paris) 72:627–637

Pittendrigh CS (1972) Circadian surfaces and the diversity of possible roles of circadian organisation in photoperiodic induction. Proc Natl Acad Sci USA 69:2734–2737

Pittendrigh CS (1981) Circadian organisation and the photoperiodic phenomena. In: Follett BK, Follett DE (eds) Biological clocks in seasonal reproductive cycles. Wright, Bristol, pp 1–35

Quay WB (1968) Individuation and lack of pineal effect in the rats' circadian locomotor rhythm. Physiol Behav 3:109–118

Quay WB (1970a) Precocious entrainment and associated characteristics of activity patterns following pinealectomy and reversal of photoperiod. Physiol Behav 5:1281–1290

Quay WB (1970b) Physiological significance of the pineal during adaptation to shifts in photoperiod. Physiol Behav 5:353–360

Rusak B (1977) The role of the suprachiasmatic nuclei in the generation of circadian rhythms in the golden hamster, *Mesocricetus auratus*. J Comp Physiol 118:165–172

Rusak B (1979) Neural mechanisms for entrainment and generation of mammalian circadian rhythms. Fed Proc 38:2589–2595

Rusak B, Morin LP (1976) Testicular responses to photoperiod are blocked by lesions of the suprachiasmatic nuclei in golden hamsters. Biol Reprod 15:366–374

Rusak B, Zucker I (1979) Neural regulation of circadian rhythms. Physiol Rev 59:449–526

Rutledge JT, Angle MJ (1977) Persistence of circadian activity rhythms in pinealectomised European starlings (*Sturnus vulgaris*). J Exp Zool 202:333–337

Sharp PJ, Follett BK (1969) The effect of hypothalamic lesions on gonadotrophin release in Japanese quail (*Coturnix coturnix japonica*). Neuroendocrinol 5:205–218

Simpson SM, Follett BK (1980) The role of the pineal and anterior hypothalamus in regulating the circadian rhythm of locomotor activity in Japanese quail. In: Nöhring R (ed) XVII Congressus Internationalis Ornithologici. Deutsche Ornithologen-Gesellschaft, Berlin, pp 435–438

Simpson SM, Follett BK (1981a) Formal properties of the circadian rhythm of locomotor activity in Japanese quail. J Comp Physiol (in press)

Simpson SM, Follett BK (1981b) Formal properties of the circadian system underlying photoperiodic time measurement in Japanese quail. J Comp Physiol (in press)

Stephan FK, Zucker I (1972) Circadian rhythms in drinking behaviour and locomotor activity of rats are eliminated by hypothalamic lesions. Proc Natl Acad Sci USA 69:1583–1586

Stetson MH (1972a) Hypothalamic regulation of gonadotrophin release in female Japanese quail. Z Zellforsch 130:411–428

Stetson MH (1972b) Hypothalamic regulation of testicular function in Japanese quail. Z Zellforsch 130:389–410

Stetson MH, Watson-Whitmyre M (1976) Nucleus suprachiasmaticus: The biological clock in the hamster? Science 191:197–199

Takahashi JS, Menaker M (1979a) Brain mechanisms in avian circadian systems. In: Suda M, Hayaishi O, Hachiro N (eds) Biological rhythms and their central mechanism. Elsevier/North Holland, Amsterdam, pp 95–109

Takahashi JS, Menaker M (1979b) Physiology of avian circadian pacemakers. Fed Proc 38 (12):2583–2588

Turek FW, McMillan JP, Menaker M (1976) Melatonin: Effects on the circadian locomotor rhythm of sparrows. Science 194:1441–1443

Underwood H (1977) Circadian organisation in lizards: The role of the pineal organ. Science 195:587–589

Underwood H (1979) Melatonin affects circadian rhythmicity in lizards. J Comp Physiol 130:317–323

Winer BJ (1971) Statistical principles in experimental design. McGraw-Hill, Igakushoin Tokyo

Yokoyama K, Oksche A, Darden TR, Farner DS (1978) The sites of encephalic photoreception in photoperiodic induction of the growth of the testes in the white-crowned sparrow, *Zonotrichia leucophrys gambellii*. Cell Tissue Res 189:441–467

Zimmerman N, Menaker M (1975) Neural connections of sparrow pineal: role in circadian control of activity. Science 190:477–479

Zimmerman N, Menaker M (1979) The pineal: a pacemaker within the circadian system of the house sparrow. Proc Natl Acad Sci USA 76:999–1003