# Photoinducible Phase for Gonadotropin Secretion Entrained to Dawn in Japanese Quail

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In previous studies (Wada, 1979, *Gen. Comp. Endocrinol.* **39**, 141–149), testicular growth and LH secretion were induced when 0.5-hr (0.5L) light pulses were given to quail kept under 8L:16D during dark periods 13–15 hr after dawn. These results strongly suggested the existence of a photoinducible phase for gonadotropin secretion. To examine whether this photoinducible phase exists in different photoperiods and whether the phase is entrained to dawn or dusk, 0.5L pulses were delivered during the dark phases of 4L:20D and 11L:13D, respectively. In both cases, LH and FSH secretion, and eventually testicular growth, were induced when 0.5L pulses were given 13 hr after dawn. In 11L:13D groups, 0.5L pulses given 19 hr after dawn also induced LH increase after 5 and 10 days of treatment along with testicular growth. The response at 19 hr could be due to phase shifting and entrainment to the 0.5L pulse, which results in coincidence of the photoinducible phase with the main photoperiod of the following day and in induction of gonadotropin release. LH and FSH release is apparently induced when light impinges on the circadian photoinducible phase entrained to daybreak.

Luteinizing hormone (LH) secretion in Japanese quail is under the control of photoperiod. When birds are transferred from short days to various long days, LH secretion can be induced if the long days are longer than 14.7 hr (Follett *et al.,* 1977). Even under short days of 8L:16D, LH secretion is inducible by a night interruption of a 30-min light pulse given 13–15 hr after onset of the main photoperiod (Wada, 1979). The photosensitive phase is evidently present and working even under short days. Photoinduction of LH release thus can be explained as the result of impinging of light over the sensitive phase.

The photoinducible phase appears to be entrained to the light–dark cycle of a day (Wada, 1979). However, it is not established whether the rhythm of the photoinducible phase is triggered by the onset of light or by its termination. If the phase is triggered by dawn, the photoinduction of LH release by light interruption of the dark period will be found 13–15 hr after onset of

lighting even though the length of the main photoperiod is increased or decreased. If the phase is triggered by dusk, LH increase by nocturnal light interruption will be found 5–7 hr after the end of the main photoperiod even if the length of the main photoperiod is changed. The present experiments were designed to resolve this issue and to confirm that the photoinducible phase is present under different photoperiods. In this experiment, follicle-stimulating hormone (FSH) was also determined by radioimmunoassay.

## MATERIALS AND METHODS

Male Japanese quail (*Coturnix coturnix japonica*) were purchased from a commercial source at the age of 3 weeks. They were kept under 4L:20D (lights on from 0900 to 1300) or 11L:13D (lights on from 0900 to 2000) according to the experimental design mentioned below for about 2 weeks before the start of the experiments. Birds were given pelletized quail food and water *ad libitum*. Several days before the start of the experiments, single birds were transferred into cages, the floor of which moved as a seesaw to record daily locomotor activity. Each deflection of the floor

triggered a microswitch and the event was recorded as a single pen deflection on a 15-channel event recorder (OPK-15, Shimadzu Denki Seisakusho Co., Kyoto). For each bird, the activity record from a single day was pasted beneath that of the previous day. A continuous activity record of 14–15 days for each animal allowed visualization of the onset of activity.

## Experiment I

A light interruption of the dark period for 30 min (0.5L) was given to quail kept under 4L:20D once per day. The time of a given pulse was different in different groups, being 2 hr apart from group to group. Control birds were not given any light pulse. Each group consisted of seven or eight birds. On Days 0, 1, 2, and 5 of treatment, blood was collected from the wing vein into heparinized capillary tubes at 1200–1300. After 10 days of treatment, the birds were killed by decapitation, trunk blood was collected, and testes were weighed. All blood samples were centrifuged and plasma or sera were stored at −20° until assay.

## Experiment II

A 30-min light interruption of the dark period was given to birds kept under 11L:13D. Other experimental designs were the same as Experiment I.

## Radioimmunoassay

Immunoreactive LH concentrations were determined according to the method described by Hattori and Wakabayashi (1979) except that the sample volume was reduced to 20 $\mu$l, $1/10$th that used in the original method. Anti-LH serum (AL-MH No. 1) was raised against chicken LH fraction IRC-2 (Gunma), and chicken LH fraction IEF-1 was used for radioiodination. LH concentration was expressed in terms of the chicken LH fraction IRC-2 (Gunma) in nanograms per milliliter.

Immunoreactive FSH concentrations were determined according to the method of Sakai and Ishii (1980) in 200-$\mu$l sample volumes in duplicate. Antiserum (RACF1S) to chicken FSH was raised in rabbits, and chicken FSH fraction (AGCHDS111135A) was used for radioiodination. FSH concentration was expressed in terms of a chicken FSH fraction AGC111A in nanograms per milliliter.

## Statistics

Student's $t$ test was employed for statistical analysis.

## RESULTS

### Experiment I

Groups of quail kept under 4L:20D were given 0.5-hr light pulses during the dark pe-

riod. Delivery time of light pulses was different in different groups. Testicular growth and serum FSH concentration after 10 days of treatment are shown in Fig. 1. In the groups where the light pulses were given at 11, 13, and 15 hr after onset of the main photoperiod, testicular growth was induced. The highest increase was found in the group that received a light interruption 13 hr after dawn. Serum FSH concentrations indicate that there was a photosensitive phase for its secretion between 11 and 15 hr after dawn (Fig. 1).

From the RIA measurement of LH concentration in each bird at 0, 1, 5, and 10 days after the first light interruption of the dark period, changes of LH concentration from Day 0 were calculated. The mean increase in each group at each day is shown in Fig. 2. One day after the first light pulse, LH release was enhanced significantly when the light pulse was given 13 hr after dawn. After 2, 5, and 10 days of treatment, considerable LH secretion was induced in the groups in which light pulses were delivered 11–15 hr after onset of the main photoperiod.
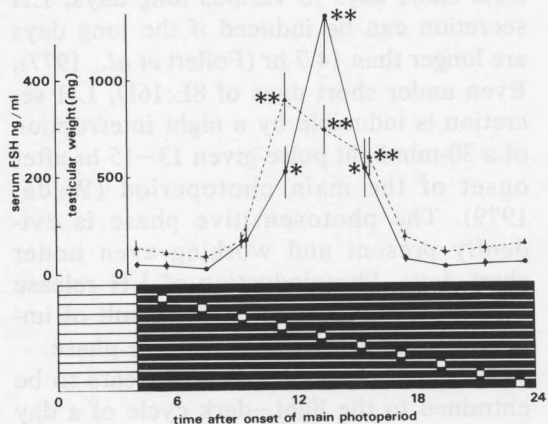


FIG. 1. Combined testicular weight (●) and serum FSH concentration (▲) in the quail 10 days after exposure to lighting schedules consisting of 4L:20D and a light pulse of 30 min indicated by a short white bar. Each point is the mean of 7 or 8 birds and vertical lines indicate standard error of the mean. Significant differences from the control group are indicated as *($P < 0.05$) and **($P < 0.01$).
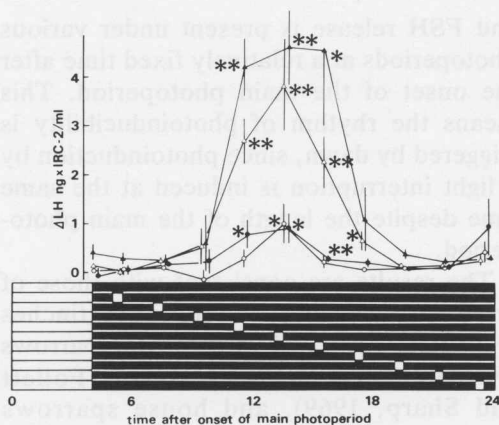
FIG. 2. Increase and decrease of plasma LH concentrations after 1 (○), 2 (●), 5 (△), and 10 (▲) days of light interruptions during the dark period. Other features are the same as in Fig. 1. *$P < 0.05$, **$P < 0.01$.

Activity records indicated that in the group in which a nocturnal light interruption was delivered 19, 21, and 23 hr after dawn, most birds underwent phase shift and entrained to the pulse photoperiods given (Figs. 5f and g). However, reconstruction of the curve of the photoinducible phase (Figs. 1 and 2) according to the circadian time in which the onset of activity is considered as the beginning of a day for the bird ("subjective" day) has no effect on the shape of the curve. In general, activity in the dark phase was noticeable in the birds kept under 4L (Fig. 5). Also in the group under 4L:1D:0.5L:18.5D, the quail tended to be active for nearly 6 hr after onset of the main photoperiod (Fig. 5b). In some birds in the groups exposed to 4L:7D:0.5L:12.5D, 4L:9D:0.5L:10.5D, and 4L:11D:0.5L:8.5D, earlier start of anticipatory activity and enhanced activity even under the dark period were obvious (Figs. 5c, d, and e).

*Experiment II*

The design in Experiment II was almost the same as in Experiment I except that the birds were kept under 11L:13D. Again a 30-min nocturnal light interruption was given during the dark period of 13D. Testicular growth and serum FSH concentra-

tions in these birds 10 days after treatment are shown in Fig. 3. Increase in testicular weight was induced in several groups, but the highest increase was found in the group exposed to 11L:2D:0.5L:10.5D. FSH concentrations suggested that two peaks were present, at 13 and at 19 hr after dawn.

LH release was induced significantly 2 days after the first treatment, if 0.5L pulses were delivered at 13 and 15 hr after dawn (Fig. 4). After 5 days, however, serum LH concentrations became elevated at 13 and also at 19 hr after dawn in this photoperiodic schedule. After 10 days of this treatment, LH concentrations attained almost similar levels in the groups in which 0.5L was given 13 to 21 hr after onset of the main photoperiod.

Activity records in each bird showed that there was no clear sign of phase shifting and entrainment to the pulse photoperiod in any group except for that exposed to 11L:12D:0.5L:0.5D (Figs. 5h, i, and j). Locomotor activity was confined mostly to the light period.

## DISCUSSION

These results together with the previous report (Wada, 1979) clearly indicate that the rhythm of the photoinducible phase for LH
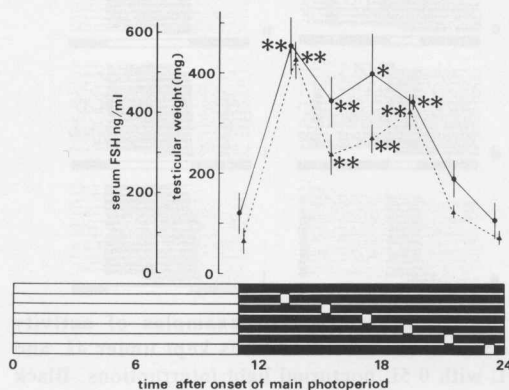


FIG. 3. Combined testicular weight (●) and serum FSH concentration (▲) in the quail 10 days after exposure to lighting schedules consisting of 11L:13D and a 0.5L light pulse indicated by a short white bar. Significant differences are indicated by *($P < 0.05$) and **($P < 0.01$).
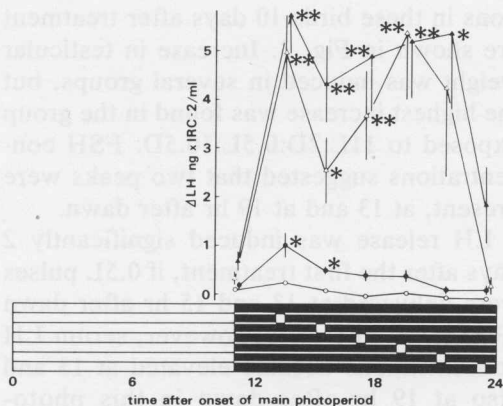
FIG. 4. Increase and decrease in plasma LH concentrations after 1 (○), 2 (●), 5 (△), and 10 (▲) days of 0.5L nocturnal light interruptions. Other features are the same as in Fig. 3. *$P < 0.05$, **$P < 0.01$.
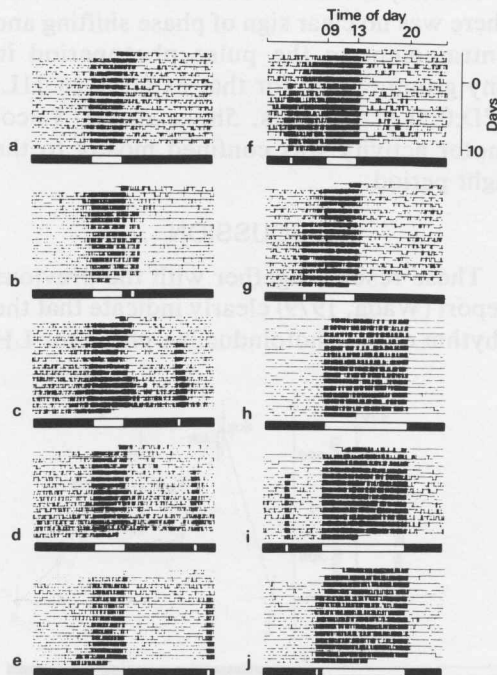


FIG. 5. Representative examples of activity records from individual birds kept under 4L and 11L with 0.5L nocturnal light interruptions. Black and white bars beneath the actogram blocks indicate dark and light period, respectively. (a) 4L:20D; (b) 4L:1D:0.5L:18.5D; (c) 4L:7D:0.5L:12.5D; (d) 4L:9D:0.5L:10.5D; (e) 4L:11D:0.5L:8.5D; (f) 4L:15D:0.5L:4.5D; (g) 4L:17D:0.5L:2.5D; (h) 11L:13D; (i) 11L:8D:0.5L:4.5D; (j) 11L:12D:0.5L:0.5D.

and FSH release is present under various photoperiods at a relatively fixed time after the onset of the main photoperiod. This means the rhythm of photoinducibility is triggered by dawn, since photoinduction by a light interruption is induced at the same time despite the length of the main photoperiod.

The results are consistent with those of previous investigations on house finches (Hammer, 1964), white-crowned sparrows (Farner, 1965), Japanese quail (Follett and Sharp, 1969), and house sparrows (Menaker, 1965; Lofts, 1975), in which gonadal development was observed. A bimodal peak of gonadal growth in interrupted night experiments has been described in several species. Follett and Sharp (1969) showed a single-peaked curve in testicular growth in Japanese quail, but in the same species, Wada (1979) described two peaks. This discrepancy is now explicable: the difference arises from the length of the main photoperiods applied in the two studies. The earlier investigators used a 6-hr photoperiod for the main light period but 8 hr was used in the more recent study. In the present experiment, there is no bimodal curve if the main light period is 4 hr but two peaks are seen if the main light period is 11 hr (Figs. 1 and 3). In 11L groups, the second peak in testicular growth was large, possibly due to the long main photoperiod. In fact appreciable development of the testes was found in the control group on 11L:13D.

The bimodal curve was not obtained when the main photoperiod was 4L. However, this does not mean that these birds underwent phase shift and entrained to the 0.5L pulse photoperiod. On the contrary, phase shifting in activity seemed to be readily inducible in the 4L group. Generally speaking, locomotor activity during the dark phase was more obvious in 4L groups than in 8L and 11L groups (see Fig. 5 of Wada, 1979, and Fig. 5 of the present experiment). In addition, the birds started to

move before the onset of light and continued to move after lights were turned off. This might be due to the short duration of the main photoperiod, 4L, which was not long enough to establish the phase relationship between the circadian locomotor activity and the environmental light–dark cycle (Aschoff, 1960; West and Pohl, 1973; Aschoff *et al.*, 1975). On the other hand, the increase in gonadotropin secretion after a light interruption indicated that phase shift might occur in 11L groups, when the 0.5L pulses were delivered 19 and 21 hr after onset of the main photoperiod. Figure 4 shows that significant LH increase was not induced in the latter half of the dark phase during the first 2 days. But after 5 days of 0.5L treatment, LH release occurred around 19 hr. This delay of response seemed to be the duration required to undergo phase shift and to entrain to the 0.5L. However, a clear phase shift in locomotor activity was not observed in these birds (Fig. 5i).

In some birds kept under 4L:9D:0.5L: 10.5D whose testes showed considerable growth, locomotor activity was greatly enhanced and the birds tended to move all day long. In quail kept under 4L:7D: 0.5L:12.5D and 4L:11D:0.5L:8.5D, earlier start of anticipatory activity was obvious. This might, at least in part, be due to an increase of the blood level of androgens. These birds had well-developed cloacal protrusions, a target organ of androgens. Testosterone is known to affect locomotor activity and free-running activity rhythms in the European starling (Gwinner, 1975).

The photoinducible phase is not pinpointed as exactly 13 hr after dawn. Figure 2 suggests that the shape of the photoinducible phase is somewhat gradual with a peak 13–15 hr after dawn. The difference in responsiveness to light could be present at a different time along the curve. The possibility is not excluded that the phase of the photosensitivity rhythm is advanced as the duration of the main photoperiod is decreased (Follett and Sharp, 1969). This photoinducibility rhythm seems to be circadian. Follett *et al.* (1974) clearly demonstrated in the white-crowned sparrow that there is a 24-hr sensitivity rhythm to a light interruption of the dark period. This rhythm ran freely for at least several cycles in constant darkness. Pentobarbiturate anesthesia inhibited LH increase after photostimulation by long days (Follett *et al.*, 1977). These results are reminiscent of the observation that in cycling female rats and hamsters, barbiturate injection before the critical period causes a 24-hr delay in ovulation (Everett and Sawyer, 1950; Everett, 1964) and in preovulatory LH release (Stetson and Watson-Whitmyre, 1977a). In castrate female rats and hamsters, a LH surge can be induced after injection or implantation of estradiol at a time similar to that of preovulatory release on the afternoon of proestrus (Norman *et al.*, 1973; Norman and Spies, 1974; Legan *et al.*, 1975; Legan and Karsch, 1975; Stetson *et al.*, 1978). Moreover, when a constant blood concentration of estradiol is attained by Silastic implantation, LH release can be synchronized with the light–dark cycle of the environment (Chazal *et al.*, 1977). These observations from mammalian and avian species lead us to propose a "gate" concept for sensitivity to light or estrogen. These sensitivity rhythms are driven by a circadian oscillator system. If the information provided by light or estrogen is present when the "gate" is open, it can affect the hypothalamo–hypophysial axis. Locomotor activity of male Japanese quail is circadian in constant dim light (Wada, 1980). The photoinducible phase in the quail may also be controlled by this or a related circadian pacemaker. In the hamster, the preovulatory LH surge is controlled by a pacemaker which also regulates locomotor activity (Stetson and Gibson, 1977; Stetson and Watson-Whitmyre, 1977b; Watson-Whitmyre and Stetson, 1977).

It is not my intention to deny the pres-

ence of a multioscillatory system in this species. Not only in rodents (Pittendrigh and Daan, 1976) but also in the starling (Gwinner, 1974), there is at least a two-oscillator system governing locomotor activity. However, at the present time, a simple external coincidence model explaining photoinduced gonadotropin release (Follett et al., 1977; Wada, 1979) appears reasonable.

In Japanese quail radioluminous implants in the hypothalamus induce testicular growth (Honma and Sakakibara, 1971; Oliver and Baylé, 1976). Yokoyama et al. (1978) have shown that direct illumination of the hypothalamus by an implanted light-conducting fiber induces testicular growth in the white-crowned sparrow. An encephalic photoreceptor appears to be involved in LH secretion in several avian species. However, a clear anatomical relationship among the oscillatory mechanism, encephalic photoreceptor, and LH−RH-producing cells is still open to be solved.

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