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Effects of Photostimulation, Castration, and Testosterone Replacement on Daily Patterns of Calling and Locomotor Activity in Japanese Quail

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To record calling (crowing) of Japanese quail continuously and automatically, an electronic device was developed. With this device, one call by Japanese quail triggers a pen deflection on a event recorder and also a count on a counter. Locomotor activity was also recorded automatically on the event recorder and the counter. Photostimulation induced calling and enhancement of activity in intact quails, and castration abolished calling and reduced locomotor activity. Silastic capsule implants containing testosterone restored both behaviors to the precastration levels in a week or so. There were daily rhythms of calling and locomotor activity in fully matured birds. These patterns also reappeared in testosterone-implanted birds, even though the release rate of the steroid hormone from the implanted capsules was expected to be constant. These results indicate that testosterone is required to induce calling and enhancement of locomotor activity and that a circadian oscillatory mechanism is possibly involved in daily rhythms of the behaviors.

Testicular androgen is required for the stimulation and maintenance of sexual behavior in male Japanese quail (Beach and Inman, 1965; Adkins and Adler, 1972; Adkins and Nock, 1976; Adkins, 1977; Adkins and Pniewski, 1978; Ottinger and Brinkley, 1978) as well as other avian species (see for review Silver, O'Connell, and Saad (1979)). These works were mainly concerned with mating behavior; hormonal control of male vocalization, which is important as an initial step of a chain of reproductive behaviors, has not yet been thoroughly studied.

In several species such as zebra finches (Arnold, 1975a, b), gulls (Terkel, Moore, and Beer, 1976), Japanese quail (Beach and Inman, 1965; Adkins and Pniewski, 1978), ring doves (Cheng and Lehrman, 1975), and pigeons (Erpino, 1969), androgens influence male vocalization. However, these works were based on observations during a certain period in a day; little is known about the whole figure of the behavior in a day based on continuous and quantitative observations and about the effect of androgens on it. Since we have experienced a different rate of calling in

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Japanese quail at different times of day, it is important to study changes of the behavior in a day.

Rhythms in locomotor activity are affected by testosterone in the European starling (Gwinner, 1975), but we do not know much about the effect of androgen on the level of locomotor activity in avian species in general. Since estrogen is known to affect locomotor activity (wheel running) in female rats, sex steroid hormones might influence locomotor activity in other species.

Thus the purposes of the present experiment are (1) to show the effect of photostimulation, castration, and testosterone replacement on calling and locomotor activity through continuous and quantitative observations and (2) to show the hourly patterns of these behaviors during a day and the effect of testosterone upon them. To achieve these purposes, an automated counting and recording system was developed.

The cloacal protrusion, which is a target organ of androgens, was also monitored as an indication of the level of the circulating androgen.

MATERIALS AND METHODS

Animals

Male Japanese quail (*Coturnix coturnix japonica*) were purchased from a commercial source at the age of 3 weeks and kept in a colony under 8L:16D (lights on from 0900 to 1700) for about 2 weeks before the start of the experiment. The birds were given pelletized quail food and water *ad libitum*.

They were then individually housed in a recording cage mentioned below to record spontaneous locomotor activity and calling (crowing) throughout the experimental period. Cloacal protrusion (gland) area of each bird was measured every 2 or 3 days with a ruler (Sachs, 1967).

Apparatus

A bird was kept in a recording cage (15 × 30 × 16 cm high), in which the floor moved as a seesaw. Each deflection of the floor triggered an installed microswitch. The cage was placed in a light-tight box (inside dimensions, 30 × 38 × 30 cm high), which was ventilated through a light-tight trap with a motor-driven fan. Inside the box illumination was provided by an overhead white fluorescent lamp through frosted glass. To prevent sudden changes of temperature when light signals were given, the lamp was covered with a water jacket with continuous water flow. The ambient temperature was 25°C. A 24-hr light–dark cycle was regulated by an external timer.

A small microphone was placed in the box, that was connected to a electronic device (Kokusai Electronics Co., Ltd., Tokyo) composed of a bandpass filter, a comparator, and a timer. Sounds with 1 to 10 kHz frequency and a certain duration are monitored by the device through the microphone and pulses are emitted. Since quail calls, composed of two or three notes, have a frequency range up to 6 or 7 kHz and continue up to 0.5 sec in duration in the third note (Cariou, 1969; Potasch, 1976), each crow can induce one pulse and other calls than crows or background noises do not.

Numbers of floor deflections and pulses by calling, respectively, were recorded by a counter (Kokusai Electronics Co., Ltd., Tokyo). Every hour, cumulative numbers of floor deflections (activity) and calls were printed automatically.

Each event was also recorded as a single pen deflection on a 15-channeled event recorder (Shimadzu Denki Keisokuki Co., Ltd., Kyoto). For each animal, the activity and calling records from a single day were pasted, respectively, beneath those of the previous day. With this method, locomotor activity and calling frequency were visualized throughout the experimental period.

Experimental Design

A preliminary experiment using two birds indicated that testosterone propionate (Sigma Chemical Co., St. Louis, Mo.) was not consistent in the induction of calling by injection (1 or 5 mg daily in sesame oil) or by subcutaneous implantation of Silastic capsules (Dow Corning Co., Midland, Mich.; the same size mentioned below), even though the cloacal protrusion developed to the mature size. Thus the free form of testosterone (Sigma Chemical Co.) was used in the experiment mentioned below.

Six birds were kept in the recording cages under short days. After 3 days (Day 0), the photoperiodic schedule was changed to long days of 16L:8D (lights on from 0900 to 0100). On Day 21, they were castrated without anesthesia between 1000 and 1200. Two weeks after castration (Day 35), Silastic tubings (3.18 mm outer diameter × 1.57 mm inner diameter; 60 mm, 2 × 30 mm in length including sealings whose thickness was the same as that of the tubing's wall), filled with crystalline testosterone and sealed with Silastic adhesive Type A at both ends, were subcutaneously implanted between 1000 and 1100. The capsules were immersed overnight in saline solution at room temperature before implantation. Two weeks after implantation (Day 49), the Silastic capsules were removed between 1000 and 1100. Implantation and removal of the implants were carried out without anesthesia within 2 to 3 min for a bird.

RESULTS

Changes in Behavioral and Morphological Variables during Photostimulation, Castration, and Testosterone Replacement

Figure 1 shows the changes in daily cumulative numbers of calls, of counts in locomotor activity, and areas of the cloacal protrusions in six

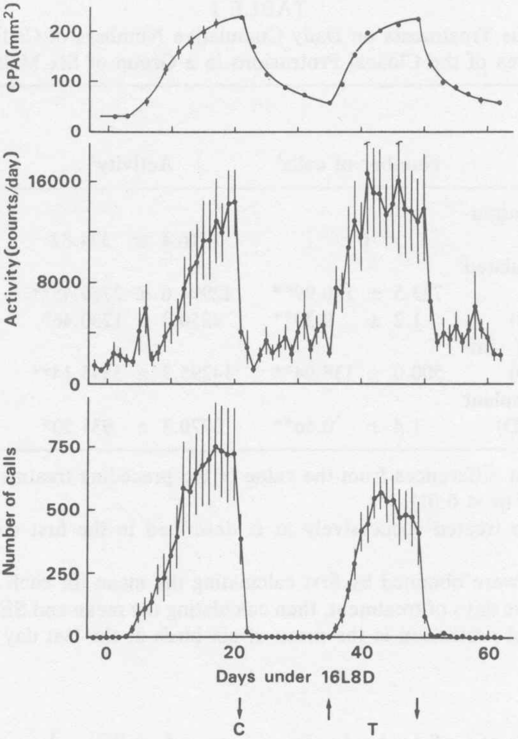

FIG. 1. Changes in numbers of calls, locomotor activity, and area of the cloacal protrusions during photostimulation, after castration and testosterone replacement. Values are the means of six birds and bars indicated standard errors of the means (SEM). The C with a down arrow indicates the day of castration, an up arrow with the T, the day of Silastic capsules of testosterone implantation, and a down arrow, the day of removal of the implants.

birds. Interindividual differences of the behaviors caused fairly large standard errors (Fig. 1 and Table 1). Long days of 16L:8D promptly induced and increased all these variables. Calling appeared 3 or 4 days after transfer to long days and the first visible growth of the cloacal protrusion was also observed at this time. Numbers of calls increased further to attain the maximal level in 2 weeks. Locomotor activity also increased after a few days of latency almost parallel to the increase of calling (Fig. 1). The maximum level of the activity was far more than what is simply calculated by doubling the activity under short days.

Castration rapidly abolished calling and also reduced locomotor activity (Fig. 1 and Table 1). During the postoperative period, calling was practically absent and locomotor activity remained reduced, although slight fluctuations in activity were observed (Fig. 1). Areas of the cloacal protrusions decreased gradually to the basal level.

TABLE 1
Effects of Various Treatments on Daily Cumulative Numbers of Calling and Activity, and Area of the Cloacal Protrusions in a Group of Six Male Quail

Treatment[a]	Number of calls[b]	Activity[b]	Area of cloacal protrusion[c] (mm^2)
Intact nonphotostimulated (8L:16D)	0	1316.4 ± 374.82	30.8 ± 0.83
Intact photostimulated (16L:8D)	723.5 ± 180.99**	12994.6 ± 2769.45**	229.0 ± 5.0**
Castrated (16L:8D)	1.2 ± 0.39**	4250.8 ± 1230.46*	57.3 ± 1.33**
Castrated and T implanted (16L:8D)	500.0 ± 138.94**	14295.3 ± 3545.14**	226.8 ± 6.36**
Castrated and T implant removed (16L:8D)	1.4 ± 0.66**	3770.3 ± 931.20*	58.3 ± 1.48**

Note. Significant differences from the value of the preceding treatment are shown with * ($p < 0.05$) or ** ($p < 0.01$).

[a] Six birds were treated successively as is described in the first column from top to bottom.

[b] These entries were obtained by first calculating the mean for each of the six birds of the last 5 successive days of treatment, then calculating the mean and SEM of these means.

[c] Area of cloacal protrusion is the mean of six birds at the last day of treatment with SEM.

Silastic implants of testosterone restored calling, locomotor activity, and area of the cloacal protrusion to the level of the intact photostimulated birds in a week or so (Fig. 1). These restored levels were quite the same as those in the intact photostimulated birds, although the number of calls was somewhat lower than that of intact birds (Fig. 1 and Table 1).

Removal of the Silastic implants resulted in reduction of calling, activity, and an area of the cloacal protrusion to the castrated level in a few days (Fig. 1).

Weight loss of the capsules during 2 weeks was estimated by weighing the capsules before implantation and after removal of the implants. Amount of released testosterone was 3.2 ± 0.32 mg (mean ± SEM, $n = 6$).

Figure 2 shows an actogram and a ''calling-gram'' of the same bird, which visualize distribution of the events in a day throughout the whole experimental period. This indicates differences in frequency of calling from time to time. The bird called vigorously a few hours after lights were turned on and also fairly frequently in the afternoon around 1700, when photostimulated and testosterone implanted. Locomotor activity was almost confined during the light phase and was somewhat sporadic in the castrated condition.

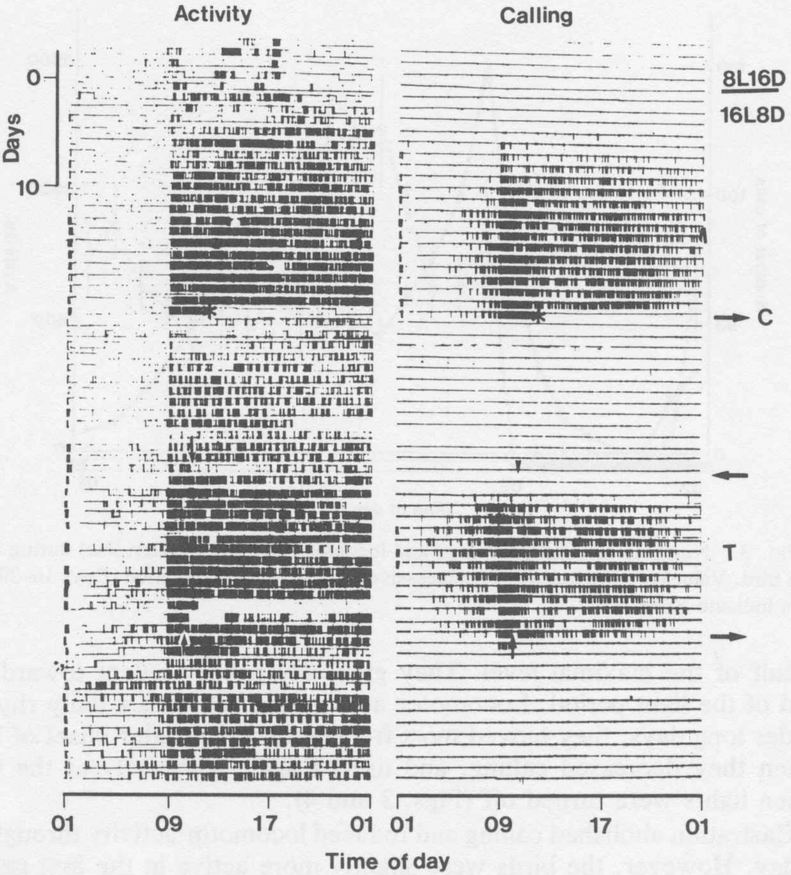

FIG. 2. Actogram and "calling-gram" of a quail throughout the whole experimental period. The lights are turned on at 0900 and turned off at 1700 under 8L:16D, and turned off at 0100 under 16L:8D. Asterisks in the blocks indicate the time of castration, arrowheads, testosterone implantation, and up arrows, removal of the implants.

Changes of Calling and Locomotor Activity during a Day

To show patterns of calling and locomotor activity in a day under long days, hourly accumulated data were analyzed. Figure 3 shows the means of numbers of calls and locomotor activity at each clock hour in one bird. The values were calculated from the last successive 5 days of photostimulation (Days 16–20). Figure 4 shows the mean numbers of calling and locomotor activity obtained from five birds. The birds began to call 3 or 4 hr before the onset of light, and gradually increased calling in number. They called most frequently during the first hour after lights were turned on. Then they decreased calling to almost one-third of the maximal level, but they called again fairly frequently around 1700, about

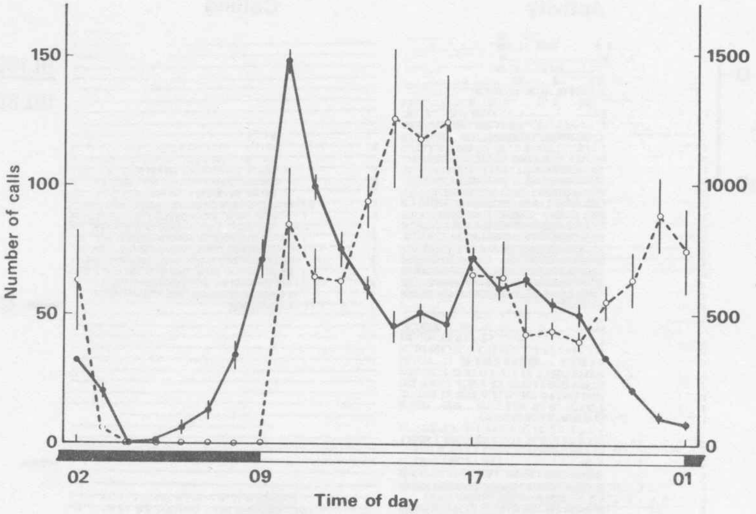

FIG. 3. Numbers of calls (solid line) and locomotor activity (broken line) during a day in a bird. Values are the means of 5 successive days of photostimulation (Days 16–20) and bars indicate SEM.

a half of the maximal level. They gradually became silent toward the end of the light period. Locomotor activity also showed a daily rhythm under long days; they moved most frequently 5–7 hr after onset of light when they decreased calling, and moved again frequently at the time when lights were turned off (Figs. 3 and 4).

Castration abolished calling and reduced locomotor activity throughout a day. However, the birds were slightly more active in the first half of the light period than in the latter half (Fig. 5a). The testosterone implants restored not only the numbers of calls and locomotor activity to the precastration level (Fig. 1), but also the hourly patterns of them (Fig. 5b). The implanted birds showed their maximal calling during the first hour after lights were turned on. The slight and broad peak around 1700 was also recovered. Locomotor activity rhythms were restored as well; three peaks were found, one at daybreak, the second around 1400–1600, and the third at the time when lights were turned off.

DISCUSSION

These results indicate that testicular androgens are required for expression of male mating calls (crows) and also for enhancement of locomotor activity in Japanese quail. Crowing in Japanese quail was described by Beach and Inman (1965) who showed castration abolished calling and testosterone replacement restored it. Adkins and Adler (1972) also showed that calling observed in quail kept under long days disappeared

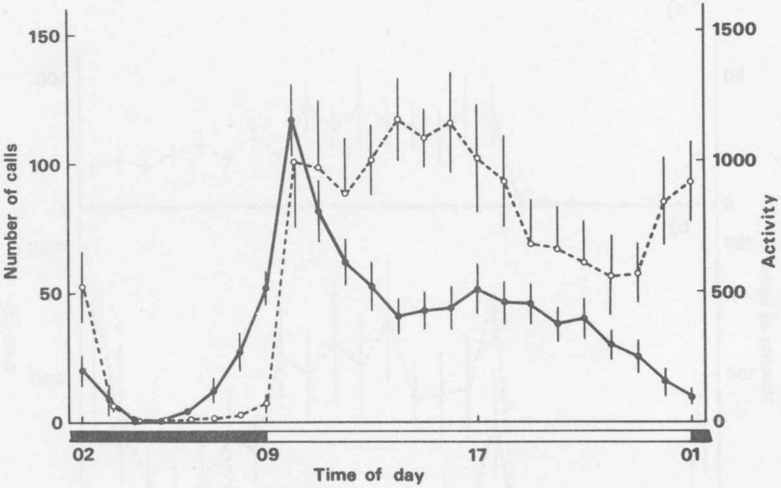

FIG. 4. Numbers of calls (solid line) and locomotor activity (broken line) during a day in five intact photostimulated birds. Values were obtained by first calculating the mean for each of five birds of the last five successive days of photostimulation (Days 16–20), then calculating the mean and SEM of these means.

when they were transferred to short days and injection of testosterone propionate restored calling in these functionally castrated birds. Generally, castration abolishes male vocalization and androgens restore it in avian species (see Silver *et al.,* 1979). However, Arnold (1975a, b) reported that in zebra finches castration did not abolish singing but the rate of singing and song tempo were reduced. Thus there might be a species difference in dependence of singing or calling on androgens.

Locomotor activity was also revealed to be under control of androgens in this species. Gwinner (1974, 1975) showed that in European starlings there was a strong positive correlation between testes sizes and durations of activity time under the constant condition. Castration suppressed an increase in activity time under the constant condition and testosterone injection restored it. In the present experiment duration of activity time in the birds was not changed, since the birds were kept under the scheduled light–dark cycle of 16L:8D. However, the amount of activity was apparently enhanced by testosterone implantation (Figs. 1 and 2). Locomotor activity might relate to maintenance of a territory. Watson (1970) showed that testosterone implantation in red grouse resulted in birds becoming more aggressive and more prone to exploratory activities that lead to enlargement of a territory.

Photostimulation is undoubtedly effective in inducing calling and enhancement of locomotor activity through activation of the pituitary–gonadal axis. Photostimulation induces LH secretion in Japanese quail (Follett,

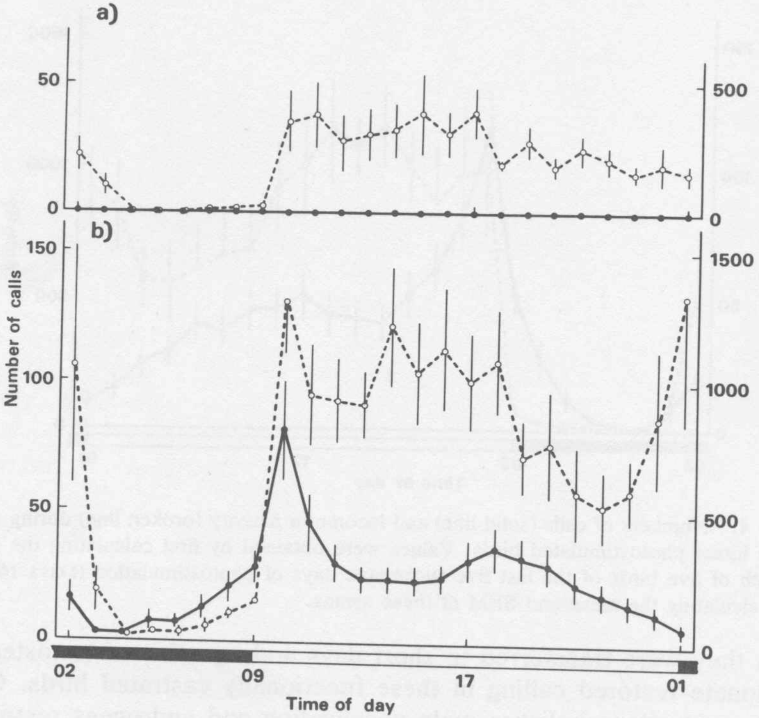

FIG. 5. Numbers of calls (solid line) and locomotor activity (broken line) during a day in five castrated (a) and castrated and testosterone implanted (b) birds. Values were obtained as in Fig. 4 from Days 30–34 (a) and Days 44–48 (b).

Davies, and Gledhill, 1977; Wada, 1979), which in turn induces testosterone secretion from the testes.

In the present experiment, the length of Silastic capsules were determined based on the results by Desjardins and Turek (1977). Table 1 indicates that the length of the capsules used was appropriate in that it released testosterone in a range of physiological concentrations. Castration and removal of Silastic implants caused abolishment of calling and reduction of locomotor activity rapidly. It suggests that testosterone is eliminated quickly from the circulation and high concentrations of testosterone are required to maintain sexual activity in this species. This differs from starlings which continue to sing for several weeks after castration (Davis, 1957) and zebra finches mentioned above (Arnold, 1975a, b). Sexual activity in mammalian species is also retained for a long time after castration, although a decrease in sexual activity is observed 1–3 weeks after castration (see for review, Larsson (1979)). On the other hand, calling gradually increases after testosterone implantation, even though the increase in blood levels of testosterone might increase to a maximal level very quickly; it has been shown that circulating estradiol increases from undetectable levels to sufficiently high levels within 1 hr after insertion of the Silastic estradiol implants and remains at these levels for several days in rats (Legan, Coon, and Karsch, 1975).

There are clear diel rhythms of calling and, although less clear, rhythms of locomotor activity in a day in intact photostimulated birds in their fully matured states. These patterns are very stable in an individual bird; compare Fig. 4 which was derived from five birds and Fig. 3 which was from a single bird. Daily fluctuations were very small in each individual, especially in calling. Testosterone replacement therapy to castrated birds restored not only calling and amount of activity, but also the rhythms of these behaviors. Morin and Zucker (1978) also observed significantly more copulations during the subjective night than during the subjective day in castrated and testosterone-capsule-implanted hamsters. Since the Silastic capsule release testosterone in a constant rate, the rhythmic expression of the behaviors might not be directly related to fluctuations of testosterone concentrations in blood. If there is a diurnal rhythmicity of the metabolic clearance rate of testosterone, circulating testosterone concentrations may change rhythmically. In fact, testosterone concentrations were higher during the subjective day than during the subjective night in the hamsters mentioned above (Morin and Zucker, 1978). However, this means more copulations observed during the subjective night is not coincident with higher concentrations of testosterone during the subjective day suggesting that copulation occurrence is not directly dependent on the high level of testosterone concentrations itself. Balthazart (1976) found high concentrations of peripheral testosterone and high sexual behaviors toward females in the early morning in domestic ducks, but other sexual activities such as copulations and aggressive behaviors toward males were almost constant during the observation period from 0800 to 1600. He suggested a direct effect of the variations of hormone levels on behavior but also argued that similar variations of behavior and testosterone levels were simply induced independently by variations in another factor such as day–night alternance. More recently, Balthazart, Massa, and Negri-Cesi (1979) found no correlation between the circulating levels of testosterone and any of the behavioral measures in Japanese quail.

Then what causes a rhythmicity of behavior, if the rhythmicity of circulating testosterone does not? In the present experiment, calling could be observed in the intact birds and testosterone implanted birds several hours before lights were turned on (Figs. 3, 4, and 5b). This indicates that the pattern of calling may be controlled by a circadian oscillatory mechanism, although testosterone itself is required for the expression of calling. In fact, calling by quail castrated and testosterone-implanted was found to be free-running under the constant condition (unpublished observation).

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