Above 24 h the values diminish slightly as a function of T. The critical number of short night signals depends on the light regime. The clock-counter system works better with very long cycles. Below 24 h it loses its efficiency. If there were a circadian element in the system (either for clock or for counter), a higher efficiency would be expected as one approaches the natural period of 24 h.

The measure of time provided by the Zeitgeber is determined by a circadian system for the induction at the larval stage while it is apparently accomplished according to an hour-glass model for diapause termination in the pupa. This change in operation during development is indicated here for the first time. Metamorphosis in holometabolous insects involves the almost total restructuring of the tissues and organs, including the central nervous system. Two situations may occur: either there is a single clock to control entry into and exit from diapause, and metamorphosis provokes a change in its operation, or induction is controlled by a larval clock operating with a circadian oscillator and diapause termination by another, anatomically different pupal clock, working according to the hour-glass model.

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1 Bünning, E., Ber. dt. bot. Ges. *54* (1936) 590.
2 Beck, S.D., Insect Photoperiodism. Academic Press, New York 1980.
3 Saunders, D.S., Insect Clocks, 2nd edn. Pergamon, Oxford 1982.
4 Horwarth, K.L., and Duman, J.G., J. exp. Zool. *219* (1982) 267.
5 Tyshchenko, V.P., and Tyshchenko, G.F., Zhur. obshch. Biol. *43* (1982) 48.
6 Tyshchenko, V.P., and Tyshchenko, G.F., Dokl. Akad. Nauk SSSR *270* (1983) 494.
7 Hamner, W.M., Science *142* (1963) 1294.
8 Follett, B.K., and Sharp, P.J., Nature *223* (1969) 968.
9 Turek, F.W., J. comp. Physiol. *92* (1964) 59.
10 Gwinner, E., and Eriksson, L.O., J. Ornithol. *118* (1977) 60.
11 Pavgi, S., and Chandola, A., Gen. comp. Endocr. *52* (1983) 126.
12 Elliott, J.A., Stetson, M.H., and Menaker, M., Science *178* (1972) 771.
13 Crocock, C.A., and Clarke, J.R., J. Reprod. Fert. *39* (1974) 337.
14 Zucker, I., Johnstons, P.G., and Frost, D., Prog. reprod. Biol. *5* (1980) 102.
15 Almeida, O.F.X., and Lincoln, G.A., Biol. Reprod. *27* (1982) 1062.
16 Nelson, R.J., Bamat, M.K., and Zucker, I., Biol. Reprod. *26* (1982) 329.
17 Whitsett, J.M., Underwood, H., and Cherry, J., Biol. Reprod. *28* (1983) 652.
18 Whitsett, J., Cherry, J., and Underwood, H., Experientia *40* (1984) 887.
19 Bernier, G., Kinet, J.M., and Sachs, R.M., The Physiology of Flowering, vol. 1. C.R.C., Florida, 1981.
20 Bonnemaison, L., Z. angew. Ent. *86* (1978) 178.
21 Underwood, H., J. comp. Physiol. *125* (1978) 143.
22 Bünning, E., Photochem. Photobiol. *9* (1969) 219.
23 Claret, J., Dumortier, B., and Brunnarius, J., C.r. hebd. Séanc. Acad. Sci., Paris *292* (1981) 427.
24 Claret, J., C.r. hebd. Séanc. Acad. Sci., Paris (D) *277* (1973) 733.
25 Brunnarius, J., and Dumortier, B., J. comp. Physiol. (A) *155* (1984) 161.
26 Pittendrigh, C.S., in: Biological Clocks in Seasonal Reproductive cycles, p. 1. Eds B.K. Follett and D.E. Follett. Scientechnica, Bristol 1981.
27 Saunders, D.S., J. Insect Physiol. *28* (1982) 305.
28 Saunders, D.S., J. Insect Physiol. *12* (1966) 569.
29 Goryshin, N.I., and Tyshchenko, V.P., Dokl. Akad. Nauk SSSR *193* (1970) 458.

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Oral melatonin produces arrhythmia in sparrows

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Summary. House sparrows, *Passer domesticus,* exhibit circadian rhythms of perch-hopping behavior. The rhythm was abolished by ad libitum administration of melatonin in the drinking water.
Key words. Passer domesticus; circadian rhythm; arrhythmia; melatonin; perch-hopping behavior.

Sparrows exhibit daily cycles of perch-hopping activity. The cycles persist in dim constant light (e.g. 40 lux) or in constant dark (DD, 0 lux) with period lengths close to 24 h and thus constitute a circadian rhythm. The circadian rhythm of perch-hopping that persists in DD is abolished by pinealectomy[2,3], lesions of the suprachiasmatic nuclei[4] or melatonin in implanted capsules[5,6]. In the experiments shown here we measured sparrows' perch-hopping behavior while we a) gave ad libitum access to water containing melatonin (lg/l) to discern whether oral melatonin could affect their rhythms, or b) implanted sparrows with 50 mm Silastic capsules containing melatonin in an attempt to replicate the prior result[5,6].

Sparrows were caught in the environs of Philadelphia. Trapped birds were maintained in a stock population in indoor aviaries (1.8 × 1.8 × 2.7 m) in LD12:12 (lights-on 6am Eastern Daylight Savings Time). Four male and 20 female sparrows contributed 48 records which form the data reported here. To record locomotor activity we placed a single sparrow in an isolation chamber. The chamber was a wooden box, painted black, which contained a cage. Two perches connected to microswitches made a single record of locomotor activity registered with Esterline Angus event recorders. The locomotor records of the sparrows were cut, pasted, and reduced photographically. Food and water was provided to the sparrows ad libitum. Light sources in the tops of the individual boxes made it possible to impose timed lighting programs on the birds. The light intensity in the boxes was 800 lux. The boxes were kept in rooms in which 90 db white noise prevented vocal interactions among the birds.

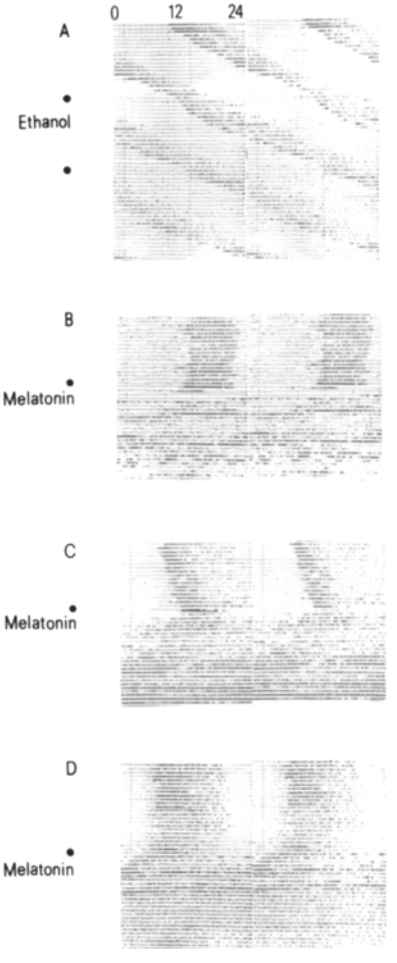
Melatonin was administered in Silastic capsules to 5 sparrows for portions of their recording periods. Capsules were made in a manner identical to those used by prior investigators – 50 mm capsules were implanted which, according to their estimates, release 40 µg of melatonin per day[5,6]. Implantations (i.p.) and removals were made under Equithesin anesthesia and involved 30 min of light exposure and handling. Controls were implanted with capsules which did not contain melatonin.

In 2 of 4 sparrows that exhibited circadian rhythms before the capsule treatments, melatonin capsules produced arrhythmia within 24 h of implantation (table, A). The third bird exhibited a relatively short freerunning period (tau = 23.6 h) and the fourth bird's rhythm became less clear when the capsule was implanted so that the period length could not be accurately measured. When the capsules were removed, circadian perch-hopping rhythms reappeared within 24 h. There was no evidence that the phase of the re-emerging rhythms extrapolated back to the phase of the rhythms before melatonin (that is, melatonin did not mask

Treatment Record number	Length	Result	Mean ± SEM
A) *Capsules*			
No capsule, before melatonin			
1	57	Freerun, 24.5	24.3 ± 0.1
2	65	Freerun, 24.4	
3	57	Freerun, 24.1	
4	8	Freerun, 24.3	
No capsule, after melatonin			
5(1)	89	Freerun, 24.6	25.2 ± 0.1
6(2)	17	Freerun, 24.9	
7(3)	25	Freerun, 25.3	
Melatonin capsule			
8(1)	91	Arrhythmic	
9(2)	81	Arrhythmic	
10	71	Freerun, 23.6	23.6
11(4)	82	Freerun, tau?	
one died			
Control capsule			
12	31	Freerun, 25.1	24.9 ± 0.2
13	20	Freerun, 25.0	
14	47	Freerun, 24.1	
15	103	Freerun, 25.7	
16	39	Freerun, 25.3	
17(2)	90	Freerun, 24.8	
18	47	Freerun, 24.9	
one died			
B) *Oral*			
Tap water			
19	28	Freerun, 24.8	24.3 ± 0.1
20	28	Freerun, 25.3	
21	28	Freerun, 24.8	
22	87	Freerun, 24.4	
23	13	Freerun, 24.1	
24	27	Freerun, 24.1	
25	27	Freerun, 24.3	
26	28	Freerun, 24.4	
27	43	Freerun, 24.3	
28	124	Freerun, 24.1	
29	29	Freerun, 23.9	
30	27	Freerun, 23.8	
Ethanol water			
31	31	Freerun, 25.3	24.9 ± 0.2
32	32	Freerun, 24.7	
33	81	Freerun, 25.0	
34	107	Freerun, 24.6	
one died			
Melatonin and ethanol water, arrhythmic			
35	84	Arrhythmic	
36	21	Arrhythmic	
37	30	Arrhythmic	
38	21	Arrhythmic	
one died			
Melatonin and ethanol water, freerun			
40	30	Freerun, 25.5	24.4 ± 0.6
41	11	Freerun, 24.3	
42	21	Freerun, 23.3	
Melatonin and ethanol water, varied effects			
43	9	Short tau?, Arrhythmic	
44	9	Some rhythm breakup	
45	27	24.6, Some rhythm breakup	
46	20	23.5, Arrhythmic	23.9 ± 0.3
47	30	23.6, Some rhythm breakup	
two died			

Table. Analysis of records of control and melatonin treated sparrows maintained in constant dark (DD). The table is divided into two parts, the capsule experiments (A) and the oral administration experiments (B). A 'record' represents the continuous recording of locomotor activity from a single sparrow treated as indicated for the length of time (in days) listed. Some sparrows were reused – numbers in parentheses indicate records from the same bird. In all, 24 sparrows contributed records to the study of which five were male and the remainder were female. 'Freerun' means that a discernible circadian rhythm was present. When a freerun was present, its period length (tau) was measured and is indicated in hours. Some birds died in the course of the studies; the number of birds that died is indicated below the treatment which immediately preceded the death. 'Arrhythmic' means that no rhythm was discernible in the record. some records were equivocal[11,43–47]. All the arrhythmic sparrows exhibited sparse activity. The three sparrows[1,2,4] for which before, during, and after melatonin records are available showed the effects of the melatonin capsule implantation or removal within 24 h. Those sparrows which responded to melatonin in the water also reacted within a cycle.

the rhythm). The birds implanted with control capsules exhibited undisrupted circadian freerunning activity. Thus, our birds replicated the arrhythmic responses to melatonin capsules that were reported by Turek and coworkers[5,6]. The one bird that freeran with an implanted melatonin capsule exhibited a shorter freerunning period than any of the controls which is expected from the results of Turek and coworkers[5,6].



Portions of perch-hopping records from four sparrows given ethanol or ethanol and melatonin in their drinking water (between the dots in A, from the dot to the end of the record in B–D). Each line is 48 h of perch-hopping activity; the lines have been arranged vertically in chronological order from top to bottom; 0 and 24 represent midnight (Eastern Daylight time), 12 represents noon; the original 24 h record has been duplicated and the duplicate record placed on the right; the 'double plotting' method permits visualization of uninterrupted freeruns. The birds were in constant dark preceding and throughout the records shown. The records were selected to show the range of patterns obtained in birds given melatonin in water. All the birds were rhythmic while drinking tap water preceding the period of melatonin administration. Bird A showed no effect of the melatonin, bird B was affected within a cycle and became arrhythmic within several days, birds C and D exhibited shortening of their circadian periods within a day and subsequently became arrhythmic within a week or two.

Melatonin in the drinking water also produced arrhythmia in sparrows (fig., table, B). The sparrows exhibited freerunning circadian perch-hopping rhythms while they were drinking tap water or tap water with ethanol. Concerning their responses, the 12 sparrows given melatonin and ethanol in their drinking water can be divided into three subgroups: a) four birds were arrhythmic, b) five birds showed a mixture of some arrhythmia and some rhythms, and c) three birds were unaffected. In all, 75% of the birds were affected by the melatonin. As with the capsules, some of the birds displayed responses to the melatonin or its removal within a cycle.

From water consumption tests we estimate the oral dose as less than 5 mg/day/bird. The response of arrhythmia requires a large dose. We note that we previously lowered sparrow body temperature and caused roosting behavior with intramuscular melatonin injections, but only with large doses (greater than 1.2 mg/bird[7]). We can conjecture about the requirement for large doses. First, sparrow melatonin synthesis is especially robust. Sparrow pineal glands have large amounts of activity of the enzymes that synthesize melatonin from serotonin – N-acetyltransferase activity increases 46-fold at night to reach levels of 3.2 nmoles/pineal/h, and sparrow pineal HIOMT is 0.7–0.8 nmoles/gland/h. Hemipineal glands from sparrows produced melatonin at rates as high as 8 ng/ml/h[8]. Possibly, large doses are needed experimentally because the response systems require high levels of melatonin. Second, when melatonin turnover was measured in other species, the rate was high (e.g. half-life of 17–23 min in rats[9]). Since we do not know the locus of the target, we do not know what effective level is required at what site.

Several further observations require comment. *First,* in both the capsule and oral experiments, melatonin may have altered the amount of activity. However, inspection of the birds' records showed reduced activity in some birds and increased activity in others. *Second,* there are apparent period changes (birds kept in the experiment longer increased period length 0.9 h, ethanol lenghened the period by 0.6 h, melatonin shortened the period 0.5–0.7 h). We advise caution in interpreting these small intraexperimental period changes because they are in the range of interexperimental variability for controls in our laboratory (24.1–24.9). *Third,* the response to melatonin may be dependent on individual sensitivity to the dose used (no response below threshold, period shortening at intermediate levels, arrhythmia at high levels). The sparrows were wild, trapped animals of varying sex, age, and history.

Oral melatonin administration has produced other effects beside arrhythmia in sparrows. The other effects are also related to pineal function – a) alteration of hamster testis size, b) advance of seasonally dependent events in white-tailed bucks, c) sedative effects in humans, and d) elevation of blood melatonin in sheep, goats and humans[10–15]. Large doses were required for most of the responses in which oral melatonin exhibited effects which correlate with pineal-related events such as reproduction, body temperature, seasonal cycles, and activity rhythms.

The similarity between melatonin administered continuously in capsules versus sporadically (rhythmically or nonrhythmically) in ad libitum oral doses may not be trivial since it implies a common final mode of action.

1 Support was provided to S. Binkley by NSF PCM 8314331.
2 Gaston, S., and Menaker, M., Science *160* (1968) 1125.
3 Binkley, S., Kluth, E., and Menaker, M., J. comp. Physiol. *83* (1972) 63.
4 Takahashi, J., in: Vertebrate Circadian Systems, p. 112. Eds J. Aschoff, S. Daan and G. Gross. Springer-Verlag, New York 1982.
5 Turek, F., McMillan, J., and Menaker, M., Science *194* (1976) 1441.
6 Hendel, R., and Turek, F., Physiol. Behav. *21* (1978) 275.
7 Binkley, S., Chronobiology, p. 472. Eds L. Scheving, F. Halberg and J. Pauly. Igaku Shoin, Ltd., Tokyo 1974.
8 Takahashi, J., Neural and endocrine regulation of avian circadian system, p. 169. Ph.D. thesis, U. Oregon 1981.
9 Gibbs, F., and Vriend, J., Endocrinology *109* (1981) 1796.
10 Pevet, P., and Haldar-Misra, C., Experientia *38* (1982) 1493.
11 Vriend, J., and Gibbs, F., Life Sci. *34* (1984) 617.
12 Vaughan, G., Pineal Res. Rev. *2* (1984) 141.
13 Kennaway, D., and Seamark, R., Aust. J. biol. Sci. *33* (1980) 349.
14 Bubenik, G., J. exp. Zool. *255* (1983) 155.
15 Waldhauser, F., Waldhauser, M., Lieberman, H., Deng, M., Lynch, H., and Wurtman, R., Neuroendocrinology *39* (1984) 307.

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Rhythmic extrusion of pheromone gland elevates pheromone release rate

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Summary. In two arctiid species, *Holomelina lamae* and *H. aurantiaca,* which rhythmically extrude and retract their abdominal tips during pheromone emission, pheromone glands contain up to three orders of magnitude more of the major component than in most Lepidoptera examined to date. Using an effluent collection technique, relatively high rates of pheromone emission were obtained from freely calling females. In contrast, volatilization rates from forcibly extruded glands were about 25 times lower for both species, suggesting that pulsation of the gland functions to increase the release rate.
Key words. Sex pheromone; pheromone emission rate; calling behavior; pheromone gland content; Arctiidae.

Many organisms transmit pulsed olfactory signals, most notably moths in the family Arctiidae[3]. Cardé[4] summarized three adaptive roles of pulsing the ovipositor and pheromone gland: 1) Higher release rates may be accomplished by 'spreading the pheromone over a larger surface area of the intersegmental membrane' in a manner analogous to 'scent marking'[5,6]. 2) 'Pulsing could create a frequency modulation of the chemical message'[3,4]. Chemical cues provide weak temporal and spatial stimuli compared to light and sound cues[7]. Signal to noise ratios may be increased and sensory adaptation reduced by introducing intermittency to the signal, thus increasing the number of potential comparisons[8]. 3) 'Pulsed message might provide an equivalent distance of downwind communication but conserve pheromone'[4]. Intermittent signals may communicate species-specific codes, positional information within the plume, and proximity to the emitter[3]. Of course, these are not necessarily mutually exclusive functions.

In this paper we address the effects of rhythmic pulsation of the abdominal tip on release rate of pheromone in two arctiid moths, *Holomelina lamae* and *H. aurantiaca.* Both emit 2-methylheptadecane as a primary component of the sex pheromone[9]. Other components, their diel periodicities in the pheromone gland and in air-borne collections, and the effects of age, weight, and wind on calling and pheromone release will be discussed elsewhere. Here we concentrate on the release rate and gland content of the most abundant component.