(SE = 2; n = 20) in diameter. It seems that these cells form a distinct subgroup of the neurosecretory cells 1, characterized by the aldehyde fuchsin method. Five to six of these cells show a large amount of the HGH-material, another six demonstrate a lower affinity for the immune reagent. This difference, however, can be expected in regard to different stages of their secretory process.

The sinus gland is homogeneously filled with granular clumps of greyish-blue HGH-material (figure 3). It is striking that a large portion of the gland is occupied by this secretory product. However, this is not uncommon, since Strolenberg et al.[5] indicate in an ultrastructural study of the sinus gland of *Astacus leptodactylus* that about 70% of the axon terminals in this neurohemal organ are filled with granulum type IV (between 100–170 nm; 49%) or type V (between 125–220 nm; 20%). In this respect we tentatively assign HGH to one of these two granulum types. To strengthen this assumption concerning the identification of HGH with one granulum type, we started an ultrastructural immunocytochemical study of the sinus gland of *Astacus leptodactylus*.

At the light microscopic level, it was possible to distinguish transverse or oblique sections through the x-organ – sinus gland – tractus by studying serial tissue sections. Some parts of that 'neurosecretory pathway' were intensively stained greyish-blue by the immunocytochemical procedure.

Note added in proof. P.P. Jaros reports in Histochemistry *63* (1979) about the immunocytochemical demonstration of the neurosecretory x-organ complex in the eyestalk of the crab *Carcinus maenas*. Using an antiserum against sinus gland extract, he was able to demonstrate by the PAP staining method the hyperglycemic hormone (HGH) and/or the black pigment dispersing hormone (BPDH) in the eyestalk of *Carcinus* on the light and electron microscopical level.

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Photoperiodic effects in the Djungarian hamster: one minute of light during darktime mimics influence of long photoperiods on testicular recrudescence, body weight and pelage colour[1]

K. Hoffmann

Max-Planck-Institut für Verhaltensphysiologie, Abt. Aschoff, D-8131 Andechs (Federal Republic of Germany), 20 February 1979

Summary. In male Djungarian hamsters (Phodopus sungorus) short photoperiods (L/D 8/16) with additional 1- or 5-min light-pulses 8 h after light-off were as effective as long photoperiods (L/D 16/8) in stimulating testicular recrudescence, increase in body weight and moult into summer pelage. The results are discussed with regard to the hypothesis that the pattern of melatonin release from the pineal gland is important in mediating photoperiodic effects in mammals.

In many mammals the annual cycle of reproductive and other functions is regulated by photoperiod and its change. The participation of the pineal gland in the mediation of photoperiodic effects has been documented[2–4].There is no consensus as to which pineal compounds are responsible for the transduction of the light effects, but melatonin seems a likely candidate since its synthesis is dependent upon external conditions of illumination[5]. Serotonin N-acetyltransferase (NAT) is assumed to be the rate-limiting enzyme in the conversion of serotonin to melatonin[5]. In all mammals studied so far, pineal NAT activity and melatonin content as well as plasma melatonin levels were found to have a marked daily cycle with high values at night and low values during the day[5,6]. As shown in studies in the laboratory rat[5,7] and in the ewe[8,9] continuous illumination suppresses the nocturnal peak of pineal NAT activity and melatonin production, and light during the normal darktime drastically reduces NAT activity within minutes, while in continuous darkness the daily cycle is maintained.

More recently, Illnerova and coworkers[10,11] have shown that in rats maintained in L/D 12/12, 1 min of light applied at about the middle of the darktime induces a rapid decline of pineal NAT activity and melatonin content as well as of plasma melatonin levels, and also prevents a subsequent rise to normal nighttime levels during the remaining darktime. Thus, such a brief exposure to light greatly alters not only the amount of melatonin produced, but also the pattern of its production and release. Since experiments in which hamsters were injected with melatonin suggest that not the amount but the temporal pattern of melatonin release might be important in the photoperiodic mechanism[3,12–15], we studied the effect of brief light exposures interrupting the darktime in the Djungarian hamster Phodopus sungorus maintained in short photoperiods. In this species strong photoperiodic effects on gonadal size and activity, body weight and pelage colour were found, and the pineal gland was shown to be involved[3,16,17].

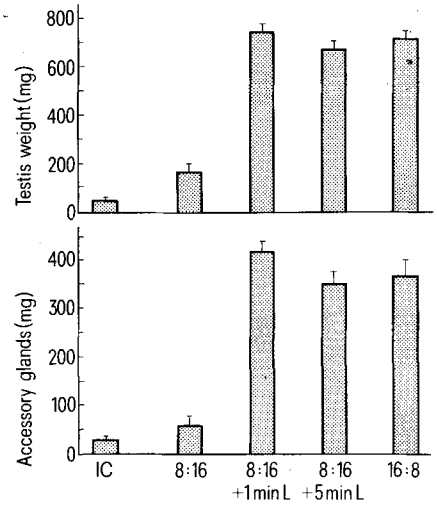
Materials and methods. On December 6, 75 adult male Djungarian hamsters (age 202–243 days) were taken from the animal house where they had been kept under natural illumination. All animals were in winter pelage and had regressed testes as ascertained by palpation. 5 groups of 15 animals each were formed, matched for age and body weight (table). 1 group (IC) was sacrificed at the beginning of the experiment. 4 groups were placed in dark rooms with constant temperature (20 ± 1 °C). In 3 of these rooms the illumination schedule was L/D 8/16; however, in 1 room the darktime was interrupted daily by 1 min of light in the middle of the dark period (L/D 8/16 + 1 min L, light 08.00–16.00 h and 23.59–24.00 h) and in another chamber the nightly lightpulse lasted 5 min, (L/D 8/16 + 5 min L, light 08.00–16.00 h and 23.55–24.00 h). A 4th group was placed into long photoperiods (L/D 16/8, light 04.00–20.00 h). Light intensities ranged 200–800 lx, depending on

position of cage in the chamber. Light was provided by fluorescent tubes (Osram L 40 W, white).

Results and discussion. After 45–47 days in these conditions, the animals were sacrificed and weight of both testes, the accessory glands (seminal vesicles, coagulating glands, ampullary glands) as well as body weight and stage of pelage colour were determined. Growth of testes and accessory glands was strongly stimulated, not only by long photoperiods, but also by interrupting the darktime with brief light pulses (figure), no significant difference could be detected between these groups. In L/D 8/16 there was a slight increase in testis and accessory gland weight as compared to the initial controls, which can be accounted for by the start of spontaneous recrudescence[3,4]. In smears from the epididymal caudae motile spermatozoa were found in all animals in long photoperiods and in short photoperiods with brief daily exposure to light during darktime, but in only 2 of the 15 males maintained in L/D 8/16. Body weight also increased by more than 30% in the hamsters maintained in long photoperiods or in short photoperiods with darktime interrupted by a light pulse (table), while it did not change in short photoperiods without such interruption. All hamsters maintained in short photoperiods with light interruptions during darktime or in long photoperiods had definitely started to moult into summer pelage, whereas in L/D 8/16 only the 2 males with most advanced testicular development showed some signs of beginning pelage change.

Body weight in male Djungarian hamsters before and after 45–47 days in different photoperiods. For symbols of photoperiodic conditions see legend of figure. Statistics (t-test): Body weight after treatment significantly higher in 8:16+1′ L, 8:16+5′ L and 16:8 than in 8:16, $p<0.001$, no significant differences between other groups

Photoperiod L/D)	N	Body weight, mean ± SE (g) Before	After	Difference
8:16	15	29.87±0.76	29.43±0.98	− 0.44±0.81
8:16+1′ L	15	30.25±0.73	43.84±1.12	+13.59±0.97
8:16+5′ L	15	30.33±0.64	40.53±1.07	+10.20±1.17
16:8	14	30.04±0.76	43.29±1.51	+13.25±1.46



Weight of both testes (above) and of accessory glands (below) from hamsters after 45–47 days in the photoperiods indicated. IC, initial controls; 8:16, 8 h light per day (light 08.00–16.00 h); 8:16+1 min L, 8 h light per day+1 min light at midnight (light 08.00–16.00 h and 23.59–24.00 h); 8:16+5 min L, 8 h light per day+5 min light at midnight (light 08.00–16.00 h and 23.55–24.00 h); 16:8, 16 h light per day (04.00–20.00 h). Means and SE are given. Statistics (U-test): Testis and accessory gland weight in 8:16+1 min L, 8:16+5 min L and 16:8 significantly higher than in 8:16 and IC (in all cases $p<0.001$); no significant differences between 8:16+1 min L, 8:16+5 min L and 16:8; testis and accessory gland weight higher in 8:16 than in IC ($p<0.002$ and <0.02).

The results show that an 8-h light-time, in connection with 1 or 5 min of light 8 h later, is equally effective in stimulating gonadal recrudescence, increase in body weight and moult into summer pelage as is a long photoperiod of 16 h duration. No report on the effects of such brief light pulses in photoperiodic studies with mammals has come to my notice. However, in golden hamsters Elliott[18] found that 1 h of light per circadian cycle may have the effect of long photoperiod, depending upon phase of the circadian cycle in which it was applied.

Taken together with the effects of 1 min illumination on the pattern of melatonin production in the rat, the results reported here support the hypothesis that the pattern of melatonin formation and release is involved in the transduction of photoperiod effects. However, the studies on light affecting melatonin synthesis were performed in the rat, a species with at best marginal photoperiodic reactions. No measurements of pineal or plasma melatonin have been reported in the hamster. Studies on NAT activity in golden hamsters have shown a daily rhythm with a peak value about 8 h after light-off[19]. It should be stressed that the amplitude of the daily cycle was only a small fraction of that observed in the rat.

Nevertheless, the findings reported here together with those mentioned in the introduction indicate that the pattern of melatonin formation may well be an important factor in the transduction of photoperiodic signals. This hypothesis is supported by the fact that surgical procedures which in the rat prevent the nocturnal rise in NAT activity, in the golden hamster abolish the effect of short photoperiods[3,4]. Studies on melatonin synthesis and its dependence on light conditions in hamsters are highly desirable.

The results given here should also caution against even brief light exposures during the dark-time, for example during feeding, maintenence work, etc., since drastic photoperiodic effects may ensue.

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