Effects of short photoperiods on puberty, growth and moult in the Djungarian hamster (*Phodopus sungorus*)

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Summary. Male Djungarian hamsters were raised from birth in either long (16L:8D) or short (8L:16D) photoperiods. In 16L:8D, testes were large (about 400 mg) and showed full spermatogenesis from 31 days of age, the cauda epididymidis contained motile spermatozoa from 35 days and the accessory glands were large and reached their final size at about 60 days of age. In 8L:16D, testes remained small (about 15 mg) and undeveloped up to an age of about 130 days; they then developed to reach normal size and full spermatogenesis. In 16L:8D body weight increased steadily up to an age of about 160 days, but in 8L:16D body weight increase was slower and reached normal values, after a plateau at about 80 days, at about 190–260 days. Most animals in 8L:16D moulted into winter pelage, and then into the summer pelage; those in 16L:8D kept the summer pelage throughout.

Introduction

Many mammals show a marked annual cycle in gonadal size and activity as well as in other functions. In a number of species it has been demonstrated that this cycle can be manipulated by photoperiod (for reviews see Aschoff, 1955; Sadleir, 1969; Chapman, 1970; Farner, Lewis & Darden, 1973; Reiter, 1974). Variations of sexual development, depending on season of birth, have also been reported (see Sadleir, 1969; Lincoln & MacKinnon, 1976).

Photoperiodic effects in the golden hamster have been studied intensively (Hoffman & Reiter, 1965; Gaston & Menaker, 1967; Elliot, 1974). In adults short photoperiods or blinding induce gonadal regression while long photoperiods instigate regrowth. Sexual development in young animals, however, is not influenced by photoperiod (Gaston & Menaker, 1967; and unpublished experiments). Reiter, Sorrentino & Hoffman (1970) found no differences of sexual development in golden hamsters blinded soon after birth and intact animals living in long photoperiods.

Similar findings have been reported for adult Djungarian hamsters. Under natural light conditions animals of this species show a marked annual cycle, not only in gonadal size but also in body weight and pelage colour (Figala, Hoffmann & Goldau, 1973; Hoffmann, 1973). In autumn testes regress to less than one-tenth of their summer size, body weight decreases by about 30%, and the moult results in a whitish winter pelage. All these changes can be instigated by photoperiodic manipulation. Short photoperiods bring about the winter state while long photoperiods cause precocious change into the summer condition (Hoffmann, 1973, 1974, 1978; Hoffmann & Küderling, 1975, 1977). Because some observations of litters born late in the breeding season suggested that photoperiod might also influence pubertal development in *Phodopus*, a systematic study of this possibility was undertaken.

Materials and Methods

All the hamsters were derived from the colony which has been maintained in this laboratory since 1968, and originated from 4 animals caught near Omsk in western Siberia, the northwest of the range of distribution (Flint, 1966). The animals belong to the subspecies *Phodopus sungorus sungorus* which, under natural illumination, changes into a whitish winter pelage in late autumn (Figala *et al.*, 1973).

Pairs were kept in plastic cages ($42 \times 28 \times 17$ cm) and received a standard diet for breeding hamsters (no. 7010, Altromin GmbH, Lage/Lippe) and water *ad libitum*. Light conditions were either natural daylight or long artificial photoperiods with 16 h light/24 h (16L:8D).

Within 48 h after parturition the adult males were removed, and the females with their litters were placed into one of two corresponding rooms which were kept at a temperature of $20 \pm 1^\circ\text{C}$. Light was provided by fluorescent tubes only, the light intensity directly above the cages being between 40 and 600 lx, depending on the position of the cage in the room. No difference in development due to differences in light intensity was observed within this range. Half of the females (and their young) were placed in 16L:8D (lights on 04:00–20:00 h) and the others were placed in short photoperiods (8L:16D; lights on 08:00–16:00 h). The young were weaned at 21 days of age, and males were placed singly in plastic cages ($29 \times 19 \times 15$ cm) in the same room and conditions of illumination. Two experiments were performed.

Experiment 1. A total of 190 males maintained in 16L:8D and 196 males maintained in 8L:16D were killed at various ages between 31 and 75 days. The body weight and pelage colour were noted and testes and accessory glands (seminal vesicles, coagulating glands, ampullary glands) were removed and weighed. Testes were fixed in Bouin, preserved in 80% alcohol, embedded in Paraplast, sectioned (usually at 10 μm) and stained in Mayer's haemalum and eosin. Smears of the contents of each cauda epididymidis were taken, diluted with 0.154 M-NaCl solution, and inspected for the presence of spermatozoa. These hamsters were weighed and inspected for pelage colour at intervals of 10 days or more until killed. In addition, 56 males maintained in 16L:8D and 34 males maintained in 8L:16D were weighed and inspected at various ages.

Experiment 2. In another group of young male hamsters testis size, body weight and pelage colour were noted from 24–34 days of age onward until 280 days age, approximately every 14 days. Testis size was determined by palpation while the animals were under halothane anaesthesia. Four stages of testis size were recognized: Stage N = testes not palpable (in adult hamsters this stage corresponds to the fully regressed condition found in late autumn and early winter; all animals with undeveloped testes in Exp. 1 were Stage N, compare Text-fig. 1a); Stage S = small testes; Stage M = testes of medium size, the cauda epididymidis does not contain spermatozoa at that stage; Stage L = large testes, corresponding to full spermatogenesis (all testes of hamsters raised in 16L:8D and older than 38 days in Exp. 1 were Stage L; see Text-fig. 1a).

Forty-two (42) males maintained in 16L:8D from birth and 43 males maintained in 8L:16D were used. Since the experiment was designed to determine whether puberty is reached at all in short photoperiods, 4 of the original 47 males raised in 8L:16D were removed from the experiment because they already showed some testicular development at 28–32 days of age, in spite of the short photoperiods.

Results

Testes. The testicular weights of the animals killed in Exp. 1 are shown in Text-fig. 1a. Males raised in 16L:8D had large testes, weighing about 400 mg, while most of those raised in 8L:16D had small testes weighing about 15 mg. Histological examination showed that the seminiferous tubular diameter was large in the 16L:8D animals and generally small in the others (Text-fig. 1b). In the testes of practically all the males kept in long photoperiods full spermatogenesis was observed, but in most males raised in short photoperiods only Sertoli cells, spermatogonia and a few spermatocytes were present. Epididymal smears showed that the cauda epididymidis of the 16L:8D animals contained motile spermatozoa from about 35–40 days of age. However, spermatozoa were detected in only 16 of 196 males kept in 8L:16D and it was these animals that had large tubular diameters and high testicular weight.

In Exp. 2 testicular development was determined by palpation. This method has been shown to be reliable in this species in our routine studies (see Hoffmann, 1973: Fig. 1). The mean values ($\pm$ s.e.m.) for 100 testes of each stage were 24.8 ± 1.8 mg for Stage N, 91.9 ± 3.9 mg for Stage S, 178.9 ± 4.7 mg for Stage M and 377.1 ± 6.9 mg for Stage L. All hamsters kept in 16L:8D ($N = 42$) and 40 or

Microscopic appearance of the testes of Djungarian hamsters. Mayer's haemalum and eosin, 5 μ m sections.

Fig. 1. Hamster raised in short photoperiods (8L:16D) and killed at 72 days.

Fig. 2. Hamster raised in long photoperiods (16L:8D) and killed at 35 days.

(Facing p. 30)

more days of age had large testes, corresponding to a state of full spermatogenesis, and remained in this condition up to the end of the experiment (Text-fig. 2a). In short photoperiods testes were not palpable (Stage N) in any of the 43 hamsters until at least 100 days of age (Text-fig. 2b). The first signs of further testicular development were observed between 116 and 164 days and full testicular size was reached at 158–207 days. These findings show that puberty finally occurs in spite of the short photoperiods, but that it is delayed for about 5 months compared to attainment of puberty at about 35 days in hamsters kept in long photoperiods.

Text-fig. 1. The effect of raising Djungarian hamsters from birth in long (16L:8D, ○) or short (8L:16D, ●) photoperiods on (a) testicular weight, (b) seminiferous tubule diameter and (c) accessory gland weight.

Accessory glands. The accessory glands showed similar weight differences between animals raised in long and in short photoperiods (Text-fig. 1c). In long photoperiods, accessory gland weight increased up to an age of about 60 days, while no increase was found in 8L:16D animals up to 75 days of age.

Body weight. The weights for the animals weighed alive and dead in Exp. 1 are shown in Table 1. Males reared and kept in long photoperiods were significantly heavier from 21 days of age onward. The values for Exp. 2 are given in Text-fig. 3(a) for the 16L:8D animals, and in Text-fig. 3(b) for the hamsters maintained in short photoperiods. In long photoperiods, body weight increased steadily until 140–180 days when a final value was reached. In short photoperiods, body weight increased

until 70–90 days, then plateaued or even declined until about 110–150 days, before increasing again to reach a final weight at 190–260 days. At 280 days of age body weight in the short-day hamsters was slightly higher (46.7 ± 0.7 g, $N = 43$) than in long photoperiods (44.0 ± 0.7 g, $N = 42$; $P < 0.005$).

Text-fig. 2. Testicular size (determined by palpation at intervals of about 14 days) of Djungarian hamsters maintained from birth in (a) long or (b) short photoperiods: N = not palpable; S = small; M = medium; L = large. (c) The pelage condition of the animals kept in short photoperiods (S = summer pelage; M = moult; W = winter pelage): all animals in the long photoperiod remained in the summer pelage.

Table 1. Body weight (mean $\pm$ s.e.m.) of hamsters raised in long (16L:8D) or short (8L:16D) photoperiods

Age (days)	16L:8D		8L:16D		Significance of difference
	No.	Body wt (g)	No.	Body wt (g)	
21–25	99	20.0 ± 0.30	76	17.3 ± 0.21	$P < 0.001$
26–30	74	23.3 ± 0.39	63	21.3 ± 0.28	$P < 0.001$
31–35	58	27.4 ± 0.45	83	21.9 ± 0.31	$P < 0.001$
36–40	60	29.3 ± 0.41	81	23.6 ± 0.26	$P < 0.001$
41–45	91	31.3 ± 0.29	94	24.5 ± 0.19	$P < 0.001$
46–50	90	32.2 ± 0.34	63	25.8 ± 0.33	$P < 0.001$
51–55	110	34.0 ± 0.29	51	26.9 ± 0.33	$P < 0.001$
56–60	93	35.4 ± 0.30	31	28.0 ± 0.52	$P < 0.001$
61–65	95	34.5 ± 0.43	60	27.4 ± 0.38	$P < 0.001$
66–70	71	35.7 ± 0.39	27	29.4 ± 0.52	$P < 0.001$
71–75	77	35.5 ± 0.43	32	29.4 ± 0.60	$P < 0.001$

Pelage colour. Up to an age of 30 days all animals in both photoperiods had summer pelage. This was maintained in the animals kept in 16L:8D throughout the experiment. In 8L:16D, however, increasing numbers of hamsters began to moult into the winter pelage. In Exp. 1 the results were

22.4% (N = 163) at 31–40 days, 54.1% (N = 157) at 41–50 days, 58.5% (N = 82) at 51–60 days, 73.6% (N = 87) at 61–70 days and 93.7% (N = 54) at 71–80 days. The pelage colour for Exp. 2 is given in Text-fig. 2(c). All the males in 8L:16D changed into the winter pelage starting between 37 and 78 days of age. The winter pelage was maintained until about 110–150 days of age and the full summer pelage was reached at 171–239 days.

Text-fig. 3. Body weights of male Djungarian hamsters kept until 280 days of age in (a) long or (b) short photoperiods. Each animal is represented by one line. ---, Body weight of adult in the winter.

Discussion

These results show that photoperiod markedly influences sexual maturation in Djungarian hamsters. In short photoperiods testes remain small and undeveloped up to an age of at least 100 days and closely resemble those found in adult males in winter, or after regression as a result of exposure to short photoperiods (Figala *et al.*, 1973; Hoffmann, 1974). The observation that in a small proportion of young males testicular development starts early in spite of short photoperiods is also paralleled by findings in adults, a small proportion of which do not react to short natural or artificial photoperiods (Figala *et al.*, 1973). The cause of this aberrant behaviour is not known. Body weight in the young males kept in short photoperiods soon reached a plateau at about 30 g, a value which closely corresponds to the body weight of adults in winter condition (Figala *et al.* 1973; Hoffmann, 1973). All the young males raised in short photoperiods and not showing early testicular development moulted into winter pelage, as do adult hamsters exposed to artificial or natural short photoperiods (Figala *et al.*, 1973; Hoffmann, 1978). In general, therefore, photoperiodic treatment of neonatal Djungarian hamsters leads to essentially the same effects as in adults, unlike the results with golden hamsters (see 'Introduction').

Maturation of the testes in juvenile *Phodopus* was not suppressed indefinitely by the short photoperiods. After about 130 days testes began to develop spontaneously and finally reached the size of those of the 16L:8D animals. Body weight and pelage colour showed similar spontaneous changes at about the same time. This corresponds to the behaviour of the adults in which spontaneous development of testes and moult into the summer pelage occur after some time in animals in which testicular regression and moult into winter pelage had been instigated by short photoperiods (Figala *et al.*, 1973; Hoffmann, 1973). In adult golden hamsters spontaneous gonadal regrowth has been reported after involution induced by short photoperiods or by blinding (Hoffman, Hester & Towns, 1965; Reiter, 1969, 1975).

A delay of puberty after exposure to short photoperiods from birth or from weaning has been reported for several species of the genus *Microtus* (Lecyk, 1963; Thibault *et al.*, 1966; Clarke & Kennedy, 1967; Grocock & Clarke, 1974). Retardation of testicular development in these species, however, was less drastic than that in *Phodopus*. Clarke & Kennedy (1967) report that the increase in body weight is greater in long than in short photoperiods. Effects of the light regimen on sexual

development have also been found for the laboratory rat which is generally considered a continuous breeder, the most extreme results being reported by Fiske (1941). Hasler, Buhl & Banks (1976) have reported that young lemmings (*Dicrostonyx groenlandicus*) raised from weaning in long or short photoperiods showed no difference in testicular development, whereas the short photoperiods induced moult into a whitish pelage in most of the juveniles. Body weights were significantly higher in short than in long photoperiods. The results of the present study of *Phodopus* and those of workers with other species demonstrate that there may be clear species differences in photoperiodic reaction and broad generalizations may be unwise.

In several mammals, including adult *Phodopus*, the pineal has been shown to participate in the photoperiodic mechanism (Reiter, 1973, 1974; Reiter, Vaughan, Vaughan, Sorrentino & Donofrio, 1975; Hoffmann, 1978). The strong photoperiodic reaction in juvenile *Phodopus* suggests that these may be useful animals for the study of pineal function in photoperiodic regulation. First experiments with neonatal *Phodopus* demonstrate that the pineal is an integral part of the photoperiodic mechanism: pinealectomy at 2 days of age partly abolished the inhibitory effect of short photoperiods on testicular development (Brackmann & Hoffmann, 1977).

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