Effect of different photoperiods on testicular weight changes in the vole, *Microtus agrestis*

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Summary. The testicular weight response of 2-week-old male voles exposed to 10, 12, 14, 15 or 16 h light (L) per day for 4 weeks and of 5-week-old males exposed from birth to 12, 13, 13·5, 14, 14·25, 14·5, 14·75, or 15 h light per day was proportional to the photoperiod: 13L or less produced little growth but 13·5L or more produced progressively heavier testes. Only 14·5L and 15L gave full spermatogenesis. 'Night-break' schedules produced a marked testicular response with 7L:8D:1L:8D and 7L:6D:1L:10D, intermediate effects with 7L:4D:1L:12D and none with 7L:2D:1L:14D. Of various skeleton photoperiods with 0·75L only 0·75L:12·5D:0·75L:10D and 0·75L:10·5D:0·75L:12D produced marked increases in testicular weight. The peak of photosensitivity appeared to be phase-shifted towards 'dawn' by reducing the initial light phase. Its position appeared to move from 16 h after 'dawn' in block photoperiods to 14 h after 'dawn' in the 'night-break' and 12 h after 'dawn' in the skeleton photoperiod experiments.

Previous experience of 16L:8D or 8L:16D had little effect on the subsequent response to skeleton photoperiods (0·5L:9D:0·5L:14D, 0·5L:13D:0·5L:10D, 0·5L:15D:0·5L:8D). Animals reared in these photoperiods only showed a positive testicular response to 0·5L:13D:0·5L:10D. The lack of response to constant darkness indicated that voles were capable of perceiving these skeleton photoperiods.

Introduction

Photoperiod is an important factor regulating reproductive activity in voles (Baker & Ranson, 1932; Clarke & Kennedy, 1967; Grocock & Clarke, 1974) as in a number of other mammals (Elliott, Stetson & Menaker, 1972; Lincoln & Davidson, 1977; Turek & Campbell, 1979) and birds (Menaker, 1965; Farner & Follett, 1966; Follett, 1973; Gwinner, 1973). Reproductive activity in 'long-day' animals can be stimulated by a long light period (e.g. 16 h) followed by a shorter dark period (e.g. 8 h). However, the photoperiodic response does not require the light phase to be continuous for 16 h since interrupting the long dark period of a short day with an appropriately placed short pulse of light will be sufficient to mimic the effects of a long day. This type of 'night-break' experiment has been used for some time in work on plant photoperiodism (Bünning, 1960) and has been shown to be effective in vertebrates, particularly birds (Farner, 1965; Menaker, 1965; Lofts & Lam, 1973; Follett, 1973; Hoffmann, 1979). 'Night-break' experiments provide some evidence for the existence of internal circadian oscillators which may be involved in the photoperiodic response. The coincidence model suggests that when light coincides with an internal circadian rhythm of photosensitivity a positive response occurs

(Pittendrigh & Minis, 1964). Pittendrigh & Daan (1976) have suggested that there are in fact two circadian oscillators which mutually interact but which are coupled separately to sunrise and sunset, thus allowing the oscillators to accommodate for seasonal changes in daylength.

Resonance experiments may provide better evidence for the existence of circadian oscillators (Follett, 1973; Follett & Davies, 1975). In this type of experiment a short photoperiod (6 or 8 h) is combined with various lengths of dark periods up to 66 h. Although the light only coincides with the photosensitive phase(s) every few days, this is sufficient to simulate the effects of long days (Hamner, 1963; Follett & Sharp, 1969; Elliott *et al.*, 1972).

Voles have been tested in resonance experiments (Grocock & Clarke, 1974) but a number of photoperiodic effects remain to be investigated, particularly with respect to the response of the animal in the field to natural daylength. Firstly, the possibility arose that there is a 'threshold' photoperiod or critical daylength above which all daylengths are stimulatory and below which they are inhibitory in terms of reproductive activity (Breed & Clarke, 1970). Secondly, a 'night-break' experiment would determine whether the vole can respond to a short pulse of light in the dark period of a short day. Thirdly, the 'night-break' experiment can be modified to produce a symmetrical skeleton photoperiod in which the initial 6 or 8 h photoperiod is reduced to the same length as the 'night-break' pulse to test whether the circadian oscillator(s) can still respond. This latter type of experiment may have relevance to the activity pattern of voles in the wild. Furthermore, the effects of previous experience may also be important in a subsequent response to any natural or artificial photoperiod.

Materials and Methods

Male voles from the colony in the Department of Human Anatomy were used. Animals were housed 3–6 to a cage after weaning. Guinea pig diet (RGP: Dixons) and water were always available. Peat moss, hay and cotton wool were provided for bedding. Photoperiods of 16 h light and 8 h dark (16L:8D) or 8 h light and 16 h dark (8L:16D) were provided in the animal house rooms by means of fluorescent tubes controlled by time clocks. Other photoperiods were set up in light boxes illuminated by fluorescent tubes and controlled by time clocks with specially designed electronic devices to give accurately timed short periods of light (e.g. 30 min). All photoperiods had the same time for lights on (09:00 h) which is subsequently referred to as 'dawn'. The patterns used are shown in Text-fig. 1.

Experiment 1: critical daylength

In Exp. 1A, male voles were raised in 16L:8D and, at 2 weeks of age, 53 were transferred to 10L:14D, 12L:12D, 14L:10D, 15L:9D or kept in 16L:8D for 4 weeks. In Exp. 1B, the males (a total of 76) were born into a photoperiod of 12, 13, 13.5, 14, 14.25, 14.5, 14.75 or 15 h light per 24 h and were killed 5 weeks later.

Experiment 2: 1 h 'night-break' experiment

A total of 76 males raised in 16L:8D were exposed, at 2 weeks of age, to 8L:16D, 7L:2D:1L:14D, 7L:4D:1L:12D, 7L:6D:1L:10D, 7L:8D:1L:8D or 16L:8D for 4 weeks.

Experiment 3: skeleton photoperiods

In Exp. 3A both the initial photoperiod and the second 'pulse' were reduced to 45 min (0.75L) light per day. The 99 males were raised in 16L:8D and at 2 weeks of age were

exposed for 4 weeks to 0.75L:6.5D:0.75L:16D, 0.75L:8.5D:0.75L:14D, 0.75L:10.5D:0.75L:12D, 0.75L:12.5D:0.75L:10D or 0.75L:14.5D:0.75L:8D.

In Exp. 3B, the light 'pulse' was reduced to 30 min (0.5L) and the photoperiods used were 0.5L:9D:0.5L:14D, 0.5L:13D:0.5L:10D or 0.5L:15D:0.5L:8D. In Group (i) the 36 males were raised in 16L:8D for 2 weeks before being transferred to one of the 3 photoperiods for 4 weeks. In Group (ii) the 42 males were raised in 8L:16D before transfer and in Group (iii) the 25 males were born into the photoperiods being tested. All animals were killed at 6 weeks of age. For comparison, Group (iv) consisted of 4 males raised in 16L:8D and transferred at 2 weeks of age to constant dark (DD) for 4 weeks, while Group (v) consisted of 3 males born and maintained in DD until 6 weeks old.

At the end of all experiments the animals were weighed and killed and the testes were removed and weighed. The testes were processed for routine light microscopy by fixation in Bouin's fluid, embedding in paraffin wax, sectioning at 5 μ m and staining with haematoxylin and eosin.

Statistical analyses were carried out to determine differences between treatments. Analysis of variance (ANOVA) was used to detect overall treatment differences and differences between treatment means were tested using Duncan's multiple range test (MRT). When *t* tests were also used this has been indicated in the text.

Results

Experiment 1: critical daylength

Photoperiods of 13 h or less prevented any significant testicular growth (Text-fig. 2). Photoperiods of 13.5 h or longer stimulated testicular growth but the amount of growth was proportional to the photoperiod. The testes of males of 5 weeks of age and exposed to 14 h light/24 h were significantly larger than those of males given 13 h or less ($P < 0.01$ by ANOVA and MRT) but significantly smaller ($P < 0.01$ MRT) than those of males given 15 h light/24 h. At 6 weeks of age, the testes of males exposed to 14 h light/24 h were significantly heavier than those of males given 12 h or less ($P < 0.02$ by *t* test only) but significantly smaller ($P < 0.01$ and $P < 0.05$ by ANOVA and MRT respectively) than those of males given 15 or 16 h light/24 h.

Experiment 2: 'night-break'

When the 1 h light pulse was positioned 6 or 8 h after the end of the initial 7-h photoperiod then the testicular response was the same as in a long photoperiod 16L:8D (Text-fig. 2). If the 1 h pulse was placed 2 h after the 7-h photoperiod then the testicular response was the same as in 8L:16D (all 'long' day treatments, i.e. Groups d, e and f, were different from 'short' day treatments, i.e. Groups a and b, $P < 0.01$, ANOVA and $P < 0.001$ MRT). When the light pulse was positioned 4 h after the initial 7 h photoperiod the response was intermediate and different ($P < 0.05$ MRT), from 'long' and 'short' day responses.

Experiment 3: skeleton photoperiods

In Exps 3A and 3B(i)–(iii) a total of 1.5 or 1 h of light was given per day. In Exp. 3A the change in testicular weight (Text-fig. 2) was similar to that in Exps 1 and 2 but the increase occurred at 12 h rather than 14 or 16 h after 'dawn.'

When the two light pulses were separated by 12.5 or 10.5 h the testes were significantly heavier than when the light pulses were separated by 14.5 h ($P < 0.01$ and $P < 0.05$ by ANOVA and MRT respectively) or by 8.5 or 6.5 h of darkness ($P < 0.01$ by ANOVA and

Text-fig. 1. Experimental design of the various photoperiods used. Each block shows the L:D ratio given daily and the duration of treatment. In Exps 1B, 3B (iii) and (v) animals were exposed to the appropriate photoperiods from birth. The numbers of animals in each group are indicated. The arrow indicates the change of photoperiod.

MRT). The testicular weights for animals born in 16L:8D (Exp. 3B(i), Text-fig. 2) and given 1 h light/day were not significantly different from those for animals given 1.5 h light per day (Exp. 3A, Text-fig. 2). Although there was an increase in testicular weight in Group 3B(i) animals when the light pulses were placed 13 h apart compared with 9 or 15 h, the differences were only just statistically significant ($P < 0.05$ only when using t tests and not ANOVA and MRT).

The results for Group 3B(ii) animals were similar to those of Group 3B(i) except that testicular weight in 0.5L:13D:0.5L:10D was significantly less ($P < 0.05$ with t test only).

Differences in the testicular response to the three photoperiods (Text-fig. 2) were more marked in animals in Group 3B(iii), with those born into 0.5L:13D:0.5L:10D having significantly larger testes ($P < 0.01$, ANOVA; $P < 0.02$, t test). Testicular weights in 0.5L:15D:0.5L:8D and 0.5L:9D:0.5L:14D in Group 3B(iii) were not significantly different

Text-fig. 2. Mean ($\pm$ s.e.m.) testicular weights of males voles subjected to the various photoperiods shown in Text-fig. 1.

from each other, but were less than those for animals in Groups 3B(i) and 3B(ii) ($P < 0.02$ for 0.5L:9D:0.5L:14D treatment, and $P < 0.02$ for 0.5L:15D:0.5L:8D treatment by t tests only).

The testicular weights of animals in Groups 3B(iv) (180 ± 37.4 mg, $N=4$) and 3B(v) (28.1 ± 3.4 mg, $N=3$) ($P < 0.02$, t test) show that previous experience of 16L:8D allows the testes to grow. It is therefore possible that some of the animals in Exp. 3B (i)–(iii) did not perceive the two short light pulses and were effectively experiencing constant darkness. However, results for animals in Group 3B(iii) which were born into 0.5L:13D:0.5L:10D and had a mean testicular weight of 210 mg suggests that this is unlikely.

Testicular histology

In general, testes heavier than 200 mg had full spermatogenesis whereas those <200 mg had variable degrees of spermatogenic development from tubules containing only spermatogonia and

Sertoli cells to those containing large numbers of germ cells but not mature spermatozoa. With long days of >14.5 h most animals had full spermatogenesis. In Exp. 2 only animals in 7L:2D:1L:14D, 8L:16D and 4 out of 10 animals in 7L:4D:1L:12D did not show full spermatogenesis. In Exp. 3 animals with testicular weights >200 mg again showed full spermatogenesis whereas those with testes <200 mg did not.

Discussion

Previous experiments on the effects of photoperiod on ovarian function (Breed & Clarke, 1970) indicated that there was a 'threshold' photoperiod of between 12 and 14 h light per day, with photoperiods of 14 h or longer stimulating and those of 12 h or less inhibiting gonadal development. Results from the present experiments indicate that 13 h of light may be the 'critical' daylength but in photoperiods longer than this testicular growth is proportional to the length of the photoperiod. Similar results have been obtained for the Japanese quail (Follett & Maung, 1978). In the present experiments it was only when the photoperiod was 14.5 h or longer that full spermatogenesis was observed in 5- and 6-week-old males. In male voles born and maintained in long days of 16L, spermatozoa first appear in the testes at 5 weeks of age but full fertility is not achieved until 6 weeks of age (Grocock, 1979).

The 'night-break' experiment (Exp. 2) indicated that, as in other species (Follett & Sharp, 1969; Murton, Lofts & Westwood, 1970; Lofts & Lam, 1973; Hoffmann, 1979), a short photoperiod, in this case 1 h of light, placed appropriately in the dark period after a short day, in this case 7 h, can induce testicular development (Text-fig. 2). An increase in testicular weight was seen when the light pulse was given 12 h after 'dawn' (7L:4D:1L:12D) but if the pulse was given 14 h after 'dawn' (7L:6D:1L:10D) testicular weight was considerably heavier (>300 mg) than in males kept in 14L:10D and comparable to those of males in 15L:9D or 16L:8D. Similar effects were observed in the skeleton photoperiod experiments when the dark period was illuminated 12 or 14 h after 'dawn'; testicular weights were >400 mg (Text-fig. 2). Illumination of the dark period 12 h after 'dawn' (0.75L:10.5D:0.75L:12D) produced heavier testes than the equivalent 'night-break' 7L:4D:1L:12D or 12L:12D photoperiod. It would therefore appear that with a continuous block of light the peak of photosensitivity occurs about 16 h after dawn. Reducing the initial photoperiod to 7 h and introducing a 1 h 'night-break' moves the peak towards 14 h after 'dawn'. A further reduction in the photoperiod to two 45-min periods moves the peak of photosensitivity nearer to 12 h after 'dawn'. Such effects have also been observed in the Japanese quail (Follett & Sharp, 1969) in which initial photoperiods of 3, 6 or 9 h followed by 15-min pulses of light caused an advancement of the photosensitive phase as the duration of the main photoperiod was decreased. The duration of the phase was, however, similar in all cases. This shifting of the photosensitive phase has also been observed under natural conditions in tree sparrows (Lofts & Lam, 1973), in which phase-shifting of the photosensitive peak occurred in the winter so that the phase did not coincide with the winter photoperiod of 11 h.

Phase-shifting in the peak of photosensitivity is not the only explanation for the difference in testicular weights of animals exposed to 7L:6D:1L:10D and 14L:10D (Exps 1 and 2). It has been observed (Follett & Sharp, 1969) that in some 'night-break' experiments the shorter light pulse may be read as 'dawn'. If this were so in the present experiments then 7L:6D:1L:10D could be considered equivalent to 18L:6D. Similar problems arise in the interpretation of the data from experiments using two symmetrical short pulses of light, 30 or 45 min (Exps 3A and 3B), because it is not clear which of the two light pulses is treated as 'dawn'.

Comparisons of the effects of previous photoperiod experience on the testicular response to two 30-min light pulses indicated that rearing the animals for 2 weeks in long or short days did not, at least in two of the light schedules, significantly alter the testicular response (Text-fig. 2).

When the two light pulses were most nearly equally spaced (0.5L:13D:0.5L:10D), animals raised in long days (16L:8D) had a significantly greater testicular weight than those reared in short days and no significant change in testicular weights was seen in voles raised on short days (8L:16D). However, animals born into the light schedules showed a significant increase in testicular weight when the dark phase was illuminated 14 h after 'dawn', thus indicating that prior entrainment to a long or short day is not required to produce a positive response to a skeleton photoperiod.

Voies in the laboratory therefore appear to be capable of responding to very short periods of light, but the question remains as to whether this mechanism is used by animals in the field. The natural habitat of voles is grassland where they live in underground burrows and surface regularly to feed. Thus they are not exposed to continuous illumination during the daytime. It seems likely that, since voles are most active at dawn and dusk, a positive testicular response will occur if light is only coincident with the photosensitive phase at either or both of these times. What remains to be shown is whether in the vole, as in the tree sparrow, the photosensitive phase is shifted according to the time of year.

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