Long-term reproductive cycles in rams after pinealectomy or superior cervical ganglionectomy

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Summary. Long-term cycles in diameter of the testes, colouration of the sexual skin and plasma concentrations of testosterone, FSH and prolactin were monitored in groups of pinealectomized (PINX), superior cervical ganglionectomized (SCGX), and control Soay rams living near Edinburgh (56°N). In Exp. 1, PINX, SCGX and control rams were kept outside for 4 years, and well defined seasonal cycles in each of the reproductive parameters were evident in all 3 groups (e.g. testosterone cycle length assessed by sine-wave analysis: 12.08 ± 0.17 , 12.39 ± 0.14 and 12.15 ± 0.10 months for PINX, SCGX and control rams respectively). Qualitative differences, however, were apparent between the groups in the timing and amplitude of the reproductive cycle. The seasonal peak in reproductive function occurred from July to September in the PINX and SCGX rams, some 2 months earlier in the year than in controls, while the amplitude of the cycle was less marked in the PINX and SCGX rams. There were no significant differences between the experimental groups in the seasonal cycle in the plasma concentrations of prolactin.

In Exp. 2, SCGX and control rams were kept indoors under an artificial environment with a 32-week light cycle and constant nutrition for 4 years. Compared to the controls, in which the reproductive changes were synchronized to the driving light cycle, the SCGX rams showed poorly defined reproductive cycles of lower amplitude and longer period (e.g. testosterone cycle length: 57.8 ± 6.1 and 32.1 ± 0.2 weeks for SCGX and control rams, respectively). There was evidence of a cycle in some of the reproductive parameters in the SCGX rams with a period close to 32 weeks during the second half of the study (e.g. testosterone cycle 32.4 ± 0.8 weeks), which was taken to indicate social induction from the neighbouring control rams. In two further short-term experiments, SCGX rams showed a decline in testicular activity in response to receiving a restricted diet (60% of controls) and an increase in testosterone secretion in response to exposure to oestrous ewes.

The overall results illustrate that PINX and SCGX rams can generate long-term synchronized cycles in pituitary and testicular activity. The animals are apparently unable to respond to changes in daylength due to the loss of the functional pineal gland but they remain competent to respond to other environmental cues such as changes in nutrition, temperature and social factors. In PINX and SCGX rams living outside under a seasonal environment, these non-photoperiodic cues are sufficient to induce a clearly defined seasonal reproductive cycle. In normal intact animals, the additional influence of the annual change in daylength results in a delay in the timing of the cycle; the consequence is the late onset of the mating season in October and November which is a characteristic feature of the Soay sheep originating from 58°N.

Keywords: seasonal reproductive rhythms; environmental cues; photoperiod; pineal gland; pituitary gland; testis

Introduction

In seasonally breeding mammals, the pineal gland relays the effects of daylength on the seasonal reproductive cycle, permitting the precise timing of the mating season (Bittman, 1984; Arendt, 1986). The pineal gland secretes melatonin during darkness, and the change in the duration of the period of melatonin secretion related to the seasonal change in daylength influences the pulsatile release of GnRH from the hypothalamus and thus affects the pituitary–gonadal axis (Lincoln & Short, 1980; Karsch *et al.*, 1984). Removal or denervation of the pineal gland blocks the reproductive responses to photoperiod, thus affecting the timing of the mating season, but these operations do not usually prevent the occurrence of cyclic changes in reproduction. The persistence of seasonal breeding after pinealectomy has been described for many species (e.g. ferret: Herbert *et al.*, 1978; sheep: Roche *et al.*, 1970; Barrell & Lapwood, 1979; Karsch *et al.*, 1984; white-tailed deer: Grumbaugh *et al.*, 1982; horse: Snyder *et al.*, 1983). The periodic changes may represent cycles which are generated endogenously as a circannual rhythm (Farner & Gwinner, 1980; Karsch & Wayne, 1988) and/or represent the influence of non-photoperiodic environmental cues which act independently of the pineal gland to affect reproduction. In the intact animal living in its natural environment, the integrative influence of many external cues including photoperiod, nutrition, temperature and social factors act together to dictate the timing of the seasonal breeding cycle (Bronson, 1988).

There have been no long-term studies of reproduction in male seasonally breeding mammals following disruption of the normal function of the pineal gland. The aim of the current study was therefore to document the changes in the activity of the pituitary–testicular axis in rams of a highly seasonal breed, in which the pineal gland had been surgically removed (pinealectomy, PINX) or functionally inactivated by removal of its sympathetic innervation (superior cervical ganglionectomy, SCGX). The long-term changes in a wide range of reproductive parameters were measured in the experimental rams living outside for 4 years, and the periodicity assessed by sine-wave and power spectral analysis. The long-term changes in reproductive activity were also analysed in SCGX rams living indoors to observe the effect of an artificial environment, and pilot experiments were conducted to establish whether SCGX rams show changes in testicular activity in response to alterations in nutrition or change in the social environment.

Materials and Methods

Animals and treatments

The animals used in these experiments were rams of the Soay breed of semi-domesticated sheep which show a marked seasonal reproductive cycle (Lincoln & Short, 1980). The animals were kept at the Dryden Field Station of the Institute of Animal Physiology and Genetics Research near Edinburgh (56°N). The operations to remove the pineal gland followed the method of Roche *et al.* (1970) and those to remove the superior cervical ganglia followed the method of Appleton & Waites (1955).

Experiment 1. Two groups of rams were studied for 4 years while housed outside. The animals lived as an all-male group in grass paddocks and had indoor shelter during the winter from December to March with supplementary feeding of hay and sheep nuts. Group 1 consisted of 4 rams which were superior cervical ganglionectomized in July 1980 at 3 months of age (SCGX rams) and 4 rams which received a sham operation at the same time (control rams). Group 2 consisted of 6 rams which were pinealectomized in July 1981 at 3 months of age (PINX rams) and 3 rams which were of similar age but received no operation (control rams). One of the PINX rams died due to an injury received while fighting in the third year of the study and the data from this animal were not included in the statistical analysis.

Throughout the 4-year study, blood samples were collected from the jugular vein of each animal every week for the measurement of the plasma concentrations of FSH, prolactin and testosterone. These three hormones were selected to illustrate the long-term cyclic changes in pituitary–testicular activity since there are large changes in the mean concentrations of these hormones during the seasonal reproductive cycle in the Soay ram (Lincoln & Short, 1980). The diameter of the testes and the intensity of the sexual skin colouration in the inguinal region were recorded every

2 weeks as additional reproductive parameters reflecting the long-term changes in testicular activity (Lincoln & Davidson, 1977).

In March, June, September and December 1983, 2 or 3 years into the experiment, blood samples were collected every 20 min for 10 h (09:00–19:00 h) from 4 animals in each group for the accurate assessment of the mean plasma concentration of FSH, prolactin and testosterone. The short-term fluctuations in the concentrations of testosterone occurring in these samples were used as an index of the pulsatile secretion of LH based on the 1:1 relationship between LH and testosterone in the peripheral blood of the ram (Lincoln & Short, 1980). For the serial blood collections the rams were moved indoors into individual animal crates on the day before the study and fitted with a jugular cannula which was kept patent using heparinized saline. In June and December 1983, blood samples were also collected hourly for 26 h for measurement of plasma melatonin.

Experiment 2. Eight rams were studied for 4 years while housed indoors; 4 of the animals were superior cervical ganglionectomized in January 1976 at 9 months of age (SCGX rams) and 4 acted as controls, one of which received the sham operation (Lincoln, 1979). The animals were initially living outside but were moved into a light-proof building in August 1976 and kept in individual pens which allowed visual and tactile contact between neighbours. They received a constant maintenance diet in pelleted form (AA6, Institute of Animal Physiology and Genetics Research, Edinburgh, UK). Artificial lighting was provided by white strip lights (140–180 lux at the animals' head height) and the photoperiod was changed between long days (16 h light:8 h darkness, 16L:8D) and short days (8L:16D) every 16 weeks. Temperature in the sheep building was not controlled and followed the normal seasonal cycle for Edinburgh. A single jugular blood sample was collected from each ram every week for hormonal measurements as in Exp. 1 and the changes in the diameter of the testes and the intensity of the sexual skin colouration were recorded every 2 weeks except for a break in 1981. The results for the first 2 years of this study have been published in detail elsewhere (Lincoln, 1979; Lincoln *et al.*, 1980, 1982).

Experiment 3. Two groups of 5 adult SCGX rams which had been housed indoors under long days (16L:8D) for at least 2 years were studied for 5 months. Group 1 received an ad-libitum diet of AA6 and their weekly food intake was monitored (control rams). Group 2 was treated in a similar way except for a period of 8 weeks when the animals were given a diet restricted to 60% by weight of the intake of the controls (food-restricted rams). A blood sample was collected from the jugular vein of all animals every week and the diameter of the testes was measured every 2 weeks.

Experiment 4. Four adult SCGX rams which had been living indoors for 3 years under an artificial lighting regimen of alternating 16-week periods of long days (16L:8D) and short days (8L:16D) were studied for 5 weeks in the middle of a period of long days when the testes were relatively enlarged but the sexual skin colouration was reduced or absent. At this time the rams which were living in individual pens with visual contact, were exposed to the novel stimulus of an oestrous ewe introduced from outside. At 1–2 day intervals over a period of 14 days, Soay ewes that had been shown to be in oestrus by using a vasectomized ram were placed in one of the animals' pens for 1–3 h to allow mating. Each of the rams was given access to an oestrous ewe on at least one occasion during the period of 14 days. Blood samples were collected from the jugular vein from each ram every 2–3 days for 32 days including a period before and after the introduction of the ewes and the intensity of the sexual skin colouration was recorded every week.

For all 4 experiments, the blood samples were heparinized, and the plasma was separated by centrifugation within 60 min of collection and stored at -20°C .

Radioimmunoassays

Hormone concentrations in the blood plasma were measured by the radioimmunoassays described previously for FSH (McNeilly *et al.*, 1976), prolactin (McNeilly & Andrews, 1974), testosterone (Corker & Davidson, 1978) and melatonin (Rollag & Niswender, 1976). The FSH assay had a lower limit of detection of 20 ng/ml plasma using NIH-FSH-S9 as reference standard and an intra-assay coefficient of variation of 5.0%. The corresponding values were 2.0 ng/ml (NIH-P-S9) and 4.0% for the prolactin assay, 0.2 ng/ml and 11.5% for the testosterone assay and 20 pg/ml and 15.0% for the melatonin assay.

Statistical analysis

The characteristics of cyclic changes in the longitudinal data were analysed in two ways: curve fitting to a best-fit sine wave, and harmonic analysis of the power spectrum. Values representing every half calendar month (Exp. 1) or every 2 weeks (Exp. 2) for each animal, or for the mean in each group, were fitted for amplitude, period and phase to an equation of the form

$$a.\sin(2t/b - c) + d$$

using the MLAB curve-fitting software at the NIH Computer Center. This yielded the period and amplitude of the cycle, an error estimate and a measure of goodness of fit (r^2) which were compared between groups by Student's *t* test or, when more than two groups were involved, by Duncan's multiple range test.

Power spectra were generated by real discrete Fourier transformation, also using MLAB; the 'power' at a given period is the square of the amplitude.

The mean annual cycle for the SCGX, PINX and control rams (Exp. 1) was determined by calculating the mean half-monthly value for each animal using data for all 4 years and then calculating all mean half-monthly values for the group $\pm$ s.e.m. The data for the 3 groups were then compared by two-way ANOVA. A similar procedure was conducted to determine the mean 32-week cycle for the SCGX and control rams in Exp. 2.

For the analysis of the hormonal data for the serial blood collections in March, June, September and December (Exp. 1), the mean hormone concentration for the 10 h period was calculated for each animal based on 20-min blood samples (31 samples) and this was used to determine the group mean $\pm$ s.e.m. The testosterone peaks in the hormone profiles were defined as any 2 consecutive values which were greater than the previous 2 values, one of which was greater than either of these values by two times the assay coefficient of variation. The results for the SCGX, PINX and control rams were compared by two-way ANOVA, and when there was a significant time *vs* group interaction the times of significant variation from the control were assessed by Newman-Keul's tests.

The results for the short-term studies (Exps 3 and 4) were analysed by ANOVA with the values compared to the pretreatment period by Newman-Keul's tests.

Results

Experiment 1: SCGX, PINX and control rams outside

The measurement of melatonin concentrations in the blood plasma collected hourly for 26 h in June and December confirmed that the ganglionectomy and pinealectomy operations were effective in permanently disrupting the normal diurnal pattern of melatonin secretion. In the SCGX and PINX rams the plasma concentrations of melatonin were low throughout the 24 h, unlike the situation in the control rams in which there was a clearly defined nocturnal increase in the levels of melatonin with a prolongation of the period of high melatonin in December closely associated with the increase in the length of darkness.

Seasonal changes in the diameter of the testes, intensity of the sexual skin flush and plasma concentrations of FSH, prolactin and testosterone were evident in all the groups of rams living outside (Figs 1 and 2). In the SCGX and PINX rams, the peak in testicular activity and FSH secretion occurred from July to September, some 2 months earlier in the year compared to the control rams; these differences were significant ($P < 0.001$, ANOVA) for testicular diameter, sexual skin flush and plasma FSH concentrations but not for plasma concentrations of testosterone (Fig. 3). The seasonal decline in testicular activity and FSH values from December to March (winter) were significantly reduced in the SCGX and PINX rams compared to the controls, indicative of a lower amplitude seasonal cycle (Fig. 3). There were no significant differences between the 3 groups in the seasonal prolactin cycle. The phase relationship between the cycle in FSH and prolactin secretion was different in the SCGX and PINX rams compared to the controls; in the two experimental groups the seasonal increase in FSH and prolactin concentrations occurred at the same time, while in the controls the increase in FSH occurred as the prolactin concentrations declined (Figs 1 and 2).

The results for the hormone analysis for the serial blood collections made in March, June, September and December confirmed that there were significant changes in the concentrations of all the reproductive hormones related to season in all groups of rams ($P < 0.001$, ANOVA, Table 1). The parameters for the SCGX and PINX rams differed from the control rams primarily in June when the operated rams had significantly higher plasma concentrations of FSH and testosterone, and a higher frequency of testosterone peaks (Table 1). There were no significant differences in the hormone profiles between the SCGX and PINX rams.

Sine-wave analysis revealed a seasonal cycle in all the reproductive parameters with a period close to 12 months, with only minor differences between the experimental and control rams (Tables 2 and 3). The correlation coefficient was generally lower for the SCGX and PINX rams compared to the control rams, reflecting a slightly less regular seasonal pattern (Tables 2 and 3). Power spectral analysis showed that the amplitude of the cycle at the 12-month harmonic was similar for the SCGX and PINX compared to the control rams for plasma prolactin, but reduced in the SCGX

Fig. 1. Long-term changes in the diameter of the testes (mean $\pm$ s.e.m.) intensity of the sexual skin flush (SF, individual values) and blood plasma concentrations of testosterone, FSH and prolactin (mean $\pm$ s.e.m.) in 4 control and 4 SCGX Soay rams living outside near Edinburgh. The values are given at half-monthly intervals from weaning until 5 years of age; the time of the surgery relative to births is shown, as well as the time of the summer and winter solstices (SS and WS).

Fig. 2. Long-term changes in the diameter of the testes (mean $\pm$ s.e.m.) intensity of the sexual skin flush (SF, individual values) and blood plasma concentrations of testosterone, FSH and prolactin (mean $\pm$ s.e.m.) in 3 control and 6 PINX Soay rams living outside near Edinburgh. The values are given at half-monthly intervals from weaning until 5 years of age; the time of surgery relative to births is shown, as well as the time of the summer and winter solstices (SS and WS).

Fig. 3. Cyclic changes in five reproductive parameters in groups of control (●), SCGX (□) and PINX (▲) Soay rams living outside near Edinburgh. Half-monthly mean values are shown based on 4 consecutive annual cycles with 4–7 rams in each group (means for the groups calculated from the means for the individuals and s.e.m. are not shown for clarity).

and PINX rams for most of the other reproductive parameters (see testicular diameter and plasma testosterone concentrations with amplitude indicated by 'power': Fig. 4).

Experiment 2: SCGX and control rams indoors

Long-term fluctuations in the reproductive parameters occurred in the SCGX rams housed indoors under the artificial 32-week light cycle (Figs 5 and 6). The long-term pattern was very different, however, from that observed in the control rams, which showed clearly defined reproductive cycles related to the imposed lighting regimen; in this group the changes were closely synchronized for the individual control rams and there were large amplitude variations in all the parameters. The reproductive changes which occurred in the SCGX rams were poorly synchronized between individuals especially during the first 2 years of the study, and were of much lower amplitude (e.g. see testicular diameter and plasma testosterone concentrations: Fig. 6).

The reproductive cycle in the SCGX and control rams related to the 32-week light cycle is summarized in Fig. 7 using the combined data for the entire 4-year study (7 light cycles). There was a significant cycle in all the reproductive parameters in the control rams ($P < 0.001$, ANOVA); maximum plasma concentrations of FSH and testicular activity occurred after exposure to short days for 8–16 weeks, while maximum plasma values of prolactin occurred under long days. There

Table 1. Summary of the changes (mean and range) in the blood plasma concentrations of FSH, prolactin, testosterone and number of testosterone peaks in control (N = 4), superior cervical ganglionectomized (SCGX, N = 4) and pinealectomized (PINX, N = 4) rams blood sampled at 20-min intervals for 10 h in March, June, September and December

	Group	March	June	September	December
FSH (ng/ml)	Control	14.3 (13.2–16.2)	26.8 (22.4–33.5)	122.9 (65.6–224.7)	32.0 (11.7–55.4)
	SCGX	47.7* (13.8–90.1)	141.4** (110.6–178.5)	46.2* (38.9–53.4)	51.1 (16.8–108.7)
	PINX	17.8 (12.5–26.5)	72.4** (49.9–102.0)	45.3* (36.6–57.6)	24.5 (16.0–30.0)
Prolactin (ng/ml)	Control	10.6 (6.7–13.3)	58.7 (28.8–90.4)	3.5 (2.3–5.5)	1.8 (0.9–2.7)
	SCGX	18.5 (12.8–32.8)	40.1 (26.6–55.2)	5.5 (1.0–9.6)	6.5 (1.9–10.5)
	PINX	13.3 (8.2–11.6)	30.7 (27.6–40.1)	6.4 (5.5–7.2)	4.0 (1.1–8.2)
Testosterone (ng/ml)	Control	1.8 (0.7–2.6)	2.4 (2.1–2.7)	16.0 (9.7–26.8)	4.4 (2.1–6.9)
	SCGX	2.8 (1.4–5.1)	6.2** (4.3–7.9)	17.1 (11.7–21.0)	1.9 (0.9–2.5)
	PINX	3.6 (2.5–3.9)	5.8* (3.0–8.2)	14.1 (11.5–17.0)	4.2 (3.2–7.1)
Testosterone peaks/10 h	Control	1.3 (1–2)	1.8 (1–2)	4.5 (3–6)	2.8 (2–5)
	SCGX	1.8 (1–3)	3.3* (3–4)	4.0 (3–6)	1.3 (1–2)
	PINX	2.0 (1–3)	3.3* (3–4)	4.3 (4–5)	2.8 (2–4)

Significantly different from control (ANOVA with Newman–Keul’s test): **P* < 0.05; ***P* < 0.01.

were only marginal changes in the reproductive parameters in the SCGX rams with a tendency for a small peak in reproductive activity at the end of the period of short days (Fig. 7).

Sine-wave analysis revealed a clearly defined cycle in all the reproductive features in the control rams with a period close to 32 weeks corresponding to the period of the driving light cycle (Table 4). The corresponding figures for the SCGX rams indicated a periodicity with a longer duration which was significantly different from those of the controls for testicular diameter, plasma testosterone and plasma prolactin (Table 4). When the long-term data were split into two parts (cycles 1–4 and cycles 4–7) it was evident that the cyclic changes in the SCGX rams underwent a transition during the experiment (Fig. 8). In the first half, the cycles had an apparent long period (probably a secular trend) and were variable between animals, and in the second half of the study the cycles had a significantly shorter period (*P* < 0.01, Student’s *t* test) and were more consistent. The changes in plasma testosterone concentrations and sexual skin colour during the second half followed a periodicity of ~32 weeks, similar to the controls, while the changes in prolactin followed a 52-week periodicity.

Power spectral analysis of the overall data confirmed the existence of a dominant harmonic at 32 weeks; this was very clearly expressed in all the reproductive parameters for the control rams but poorly expressed in the data for the SCGX rams (Fig. 9).

Table 2. Summary of sine-wave analysis of reproductive data for control (N = 4) and SCGX rams (N = 4) living outside for 4 years, showing the period of the cycle and correlation coefficients for goodness of fit

	Periods of cycle (months)		Correlation (r^2)	
	Control	SCGX	Control	SCGX
Testis diameter	12.12 ± 0.05	11.87 ± 0.09	0.633 ± 0.037	0.440* ± 0.052
Sexual skin flush	11.92 ± 0.08	12.01 ± 0.16	0.372 ± 0.031	0.4326 ± 0.017
Testosterone	12.20 ± 0.05	12.39 ± 0.14	0.390 ± 0.027	0.346 ± 0.037
FSH	12.38 ± 0.17	11.89* ± 0.07	0.445 ± 0.050	0.320 ± 0.075
Prolactin	12.40 ± 0.07	12.61* ± 0.05	0.645 ± 0.017	0.364** ± 0.048

Values are mean $\pm$ s.e.m.

Significantly different from corresponding control (Student's *t* test):

* $P < 0.05$; ** $P < 0.01$.

Table 3. Summary of sine-wave analysis of reproductive data for control (N = 3) and PINX rams (N = 5) living outside for 4 years, showing the period of the cycle and correlation coefficients for goodness of fit

	Periods of cycle (months)		Correlation (r^2)	
	Control	PINX	Control	PINX
Testis diameter	11.73 ± 0.06	11.60 ± 0.11	0.540 ± 0.009	0.287* ± 0.040
Sexual skin flush	11.58 ± 0.11	11.61 ± 0.35	0.446 ± 0.035	0.400 ± 0.023
Testosterone	12.03 ± 0.14	12.08 ± 0.17	0.329 ± 0.026	0.268 ± 0.029
FSH	12.12 ± 0.24	12.10 ± 0.10	0.431 ± 0.032	0.336 ± 0.026
Prolactin	11.99 ± 0.19	12.34 ± 0.08	0.642 ± 0.053	0.408** ± 0.021

Values are mean $\pm$ s.e.m.

Significantly different from corresponding control (Student's *t* test):

* $P < 0.05$; ** $P < 0.01$.

Experiment 3: SCGX rams and the effect of nutrition

The SCGX rams housed indoors under long days with a constant diet showed only minor changes in testicular diameter with the testes remaining relatively large. The restriction of food to 60% of the normal intake for 8 weeks in one group of SCGX rams resulted in a significant decline

Fig. 4. Power spectral analysis of the cyclic changes in the diameter of the testes (a, b) blood plasma concentrations of testosterone (c, d) in groups of control (---) and SCGX (—) (left panels) and control (---) and PINX (—) (right panels) Soay rams living outside near Edinburgh from weaning to 5 years of age. Note the occurrence of a dominant harmonic with a period close to 12 months which is generally less conspicuous (reduced power) in the experimental animals.

in testicular diameter and plasma concentrations of testosterone ($P < 0.05$, ANOVA, Fig. 10). These changes were rapidly reversed when the animals were given food *ad libitum*.

Experiment 4: SCGX rams and the effect of a sexual stimulus

The long-term SCGX rams were capable of mating with ewes resulting in pregnancy. The introduction of an oestrous ewe provoked normal sexual behaviour from the accompanying ram including flehmen and induced a marked increase in overt aggression between rams in neighbouring pens. The introduction of oestrous ewes on several consecutive days resulted in an increase in the plasma concentration of testosterone in the group of SCGX rams and stimulated the development of the sexual skin flush in all the animals within 1–7 days (Fig. 11).

Discussion

These results show clearly that cyclic changes in pituitary and testicular activity occur in rams in which there is no functional pineal gland. This was most evident for the SCGX and PINX rams kept outside in a markedly seasonal environment in which there was a well defined 12 month periodicity in all the reproductive parameters with the cyclic changes well synchronized to the time of year. The persistence of long-term reproductive cycles was less evident for the SCGX rams housed indoors in the artificial environment with a 32-week lighting regimen and a constant diet. Under these conditions the cycles were poorly defined and irregular, and only in the second half of the 4 year study was there evidence of synchronized changes in some of the reproductive parameters with a fixed periodicity. In both the outdoor and indoor studies the reproductive

Fig. 5. Long-term changes in the diameter of the testes (mean $\pm$ s.e.m.), intensity of the sexual skin flush (SF, individual values) and the plasma concentration of testosterone, FSH and prolactin (mean $\pm$ s.e.m.) in 4 control and 4 SCGX Soay rams living indoors under an artificial light regimen of alternating 16-week periods of short days (8 h light:16 h darkness, SD) and long days (16 h light:8 h darkness, LD). The values are given at intervals of 2 weeks from the time the animals were introduced into the artificial conditions at 16 months of age for over 4 years; the time of the surgery is indicated ($\blacktriangledown$).

patterns in the experimental groups were clearly different from the patterns expressed by the corresponding control animals. This illustrates that the pineal gland plays an important role in the physiological timing process regulating the seasonal reproductive cycle but is not exclusively involved.

Annual changes in daylength, nutrition, temperature and social factors are all features of the outside environment which may interact to influence the seasonal reproductive cycle (Sadleir, 1969;

Fig. 6. Long-term changes in the three aspects of testicular activity in 1 control and 2 SCGX Soay rams living indoors under an artificial lighting regimen of alternating 16-week periods of short days (8 h light:16 h darkness, SD) and long days (16 h light:8 h darkness, LD) for over 4 years. SF, sexual flush, scored on a scale 0–5.

Fig. 7. Cyclic changes in five reproductive parameters in groups of control (●) and SCGX (□) Soay rams housed indoors under an artificial 32-week lighting regimen. Two-weekly mean values are shown based on 7 consecutive cycles with 4 rams in each group (means for the groups calculated from means for the individuals and s.e.m. are not shown for clarity).

Table 4. Summary of sine-wave analysis of reproductive data for control (N = 4) and SCGX rams (N = 4) housed indoors under a 32-week light cycle for 4 years, showing the period of the cycle and correlation coefficients for goodness of fit

	Periods of cycle (months)		Correlation (r^2)	
	Control	SCGX	Control	SCGX
Testis diameter	32.7 ± 0.1	87.1** ± 1.2	0.797 ± 0.052	0.196* ± 0.015
Sexual skin flush	32.8 ± 0.2	43.2 ± 8.4	0.467 ± 0.032	0.171** ± 0.036
Testosterone	32.1 ± 0.2	57.8* ± 6.1	0.614 ± 0.022	0.168** ± 0.009
FSH	32.9 ± 0.1	52.5 ± 26.5	0.523 ± 0.020	0.272** ± 0.095
Prolactin	31.7 ± 0.1	60.8* ± 8.9	0.632 ± 0.034	0.202** ± 0.056

Values are mean $\pm$ s.e.m.

Significantly different from corresponding control (Student's *t* test):

* $P < 0.05$; ** $P < 0.01$.

Fig. 8. Length of the reproductive cycle in 4 control and 4 SCGX Soay rams calculated by sine-wave analysis for the first half (A, cycles 1–4) and second half (B, cycles 4–7) of the study in which the rams were exposed to an artificial 32-week lighting regimen for 7 consecutive cycles.

Bronson, 1985, 1988; Gilmore & Cook 1987). The effects of daylength on reproduction are known to be mediated through the daily pattern of melatonin secretion from the pineal gland while the effects of the other environmental factors do not apparently involve this control system (Martin, 1984; Karsch & Wayne, 1988). The inactivation of the pineal gland by SCGX or PINX therefore

Fig. 9. Power spectral analysis of the cyclic changes in the diameter of the testes (a) and in plasma concentrations of testosterone (b) in 4 control (---) and 4 SCGX Soay rams (—) housed indoors under an artificial 32-week lighting regimen for 7 consecutive cycles. Note the occurrence of a dominant harmonic with a period of 32 weeks which is very much less conspicuous (see insert for testis diameter) or absent in the SCGX rams.

Fig. 10. Changes in diameter of the testes and blood plasma concentrations of testosterone (mean $\pm$ s.e.m.) in two groups of 5 SCGX Soay rams housed indoors under long days (16 h light:8 h darkness); rams in (a) were given a restricted diet for 8 weeks as indicated, and those in (b) were maintained on a constant diet *ad libitum*. * $P < 0.05$ compared with pretreatment value (ANOVA with Newman-Keul's t test).

Fig. 11. Changes in intensity of the sexual skin flush (SF, individual values) and blood plasma concentration of testosterone (mean $\pm$ s.e.m.) in 4 SCGX Soay rams living indoors under long days (16 h light:8 h darkness). The animals were living in individual pens in visual contact and an oestrous ewe was placed in one of the pens for 1–3 h every 1–3 days over a period of 2 weeks as indicated. * $P < 0.05$ compared with pretreatment value (ANOVA with Student's t test).

specifically blocks the reproductive responses to changes in daylength without necessarily impairing the responses to other environmental factors such as nutrition and social cues. This is consistent with the results for the SCGX rams housed indoors which failed to respond in the normal way to the imposed 32-week light cycle but which were shown in the pilot studies to be able to respond normally to a decrease in nutrition or exposure to a sexual stimulus. In the absence of the predominant influence of daylength, the influence of the other environmental factors may thus govern reproductive changes; this could explain the occurrence of the conspicuous seasonality in the SCGX and PINX rams kept outside which were exposed to changes in all the important environmental cues.

Variation in nutrition is an important factor of the natural environment which directly influences the seasonal reproductive cycle. In rams, an increase in protein in the diet causes an increase in the secretion of LH and testosterone and prolonged growth of the testes; these changes are reversed by a reduction in the quality of the diet (Oldham *et al.*, 1978; Sutherland & Martin, 1980; Ritar *et al.*, 1984). For sheep adapted to lower latitudes, such as the Merino, annual change in the quality of the grazing is apparently the predominant factor dictating the seasonal changes in testicular activity (Masters & Fels, 1984; Martin, 1984). In the current study, the rams kept outside experienced seasonal changes in nutrition with the best grazing available from June to August. All groups of animals increased in body weight in summer and decreased in body weight in winter and the seasonal changes in body weight closely paralleled the changes in testicular diameter (see Ebling & Lincoln, 1987). For the indoor animals, a constant diet was provided and under these conditions the SCGX rams did not show marked cyclic changes in the size of the testes. Furthermore, the exposure of a group of SCGX rams to a period of food restriction lasting 8 weeks caused a decrease in testosterone secretion and testicular activity which was rapidly reversed following the reintroduction of the full diet. Based on these results it is very probable that the seasonal change in nutrition was a major factor which influenced the 12-month periodicity in the reproductive characteristics of the SCGX and PINX rams living outside.

Variations in temperature may have also contributed to the seasonality of the experimental animals since there are relatively large fluctuations in temperature from summer to winter in Edinburgh at 56°N. Prolactin secretion in ruminants is particularly responsive to ambient temperature with high temperatures stimulating and low temperatures inhibiting the hormone release (Tucker & Wettemann, 1976; Mori *et al.*, 1985). In the SCGX and PINX rams living outside there was a conspicuous annual cycle in the plasma concentrations of prolactin which was not significantly different from that observed in the control rams. There was no cycle in prolactin secretion in the SCGX rams housed indoors related to the 32-week lighting regimen in contrast to the control rams; however, there was evidence of cyclic changes in prolactin with a period of 52 weeks. Since the temperature in the animal house was not controlled and varied seasonally with the outside temperature, it is probable that the changes in temperature influenced the secretion in prolactin in the animals in which the dominant effect of photoperiod had been removed.

Changes in the social and sexual environment of the rams may have also contributed to the changes in the reproductive patterns in the experimental animals. The introduction of ewes and the stimulus of mating can cause an acute increase in the secretion of LH and testosterone in rams and housing adult rams in close proximity to ewes can result in long-term stimulatory effects on testicular activity (Illius *et al.*, 1976; Sanford & Yarney, 1983). Similar stimulatory effects can occur in SCGX rams as illustrated in the present study, in which the introduction of oestrous ewes to a group of indoor rams led to an increase in testosterone secretion and the full development of the sexual skin colouration within a few days. The SCGX and PINX rams involved in the long-term study were never in direct contact with ewes although these were present in surrounding fields. These experimental rams were in close contact, however, with the control rams which provided a changing social environment since these animals showed marked changes in sexual and aggressive behaviour related to the rutting season (Lincoln & Davidson, 1977). The stimulus from the control rams may thus have influenced the experimental rams although it cannot readily account for the seasonal resurgence of reproductive activity in these rams since this began earlier in the year compared to the controls. Social induction may have been more evident for the SCGX rams living indoors since these were penned side-by-side with the control rams. In the second half of the 4-year study on these animals, a low-amplitude cycle in the plasma levels of testosterone and the intensity of the skin colouration was observed with a period of about 32 weeks. This could be interpreted as a direct influence of the artificial lighting regimen, but it more probably reflects the inductive influence of the control rams which responded to the changes on photoperiod with overt changes in behaviour and the production of pheromones. Comparable studies in ewes which involved housing PINX or blind animals in close proximity to intact ewes or rams have also provided evidence that social cues can influence the timing of the seasonal reproductive cycle (Karsch *et al.*, 1981; Karsch & Wayne, 1988).

In conclusion, the study supports the view that multiple environmental factors, including daylength, nutrition, temperature and social stimuli, act to induce or entrain the seasonal reproductive cycle in rams living outside in a natural environment. In SCGX and PINX rams in which the response to daylength is ablated, the other environmental cues still produce a well defined reproductive cycle synchronized to the seasons. Seasonal changes in nutrition appear to be particularly important in dictating the seasonality in the functionally pinealectomized animals, while it is likely that in the normal intact rams the effects of nutrition and photoperiod interact to dictate the timing of the mating season. SCGX and PINX rams living outside show a seasonal cycle in testicular activity which closely parallels the seasonal changes in the quality of the diet while intact rams show a seasonal cycle phase-shifted some 2 months later in the year. The response to changes in daylength can be considered critically important since it allows the late timing of the seasonal reproductive cycle with the mating season in October and November. The late onset of the mating season is a characteristic feature of a northern breed such as the Soay sheep which in its natural habitat on the Isle of St Kilda at 58°N has a lambing season in April coinciding with the beginning of the summer grazing (Grubb & Jewell, 1973).

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