Effect of light entrainment and temperature on the reproductive cycle in the male hedgehog (*Erinaceus europaeus*)

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The role of photoperiod in the entrainment and synchronization of the reproductive cycle of male hedgehogs, seasonal breeders and hibernating mammals, was investigated. Groups of adult hedgehogs were either maintained outdoors (controls, n = 6) or submitted to accelerated 6-month artificial light regimens under constant ambient temperatures (20 ± 2°C versus 5 ± 1°C) in light-proofed rooms. The daily duration of light was varied sinusoidally to produce an amplitude change from 8 h (winter solstice) to 16 h (summer solstice) during the 6-month light cycle. Animals were transferred from outdoors to a high ambient temperature (20 ± 2°C) and submitted to accelerated 6-month light regimens at two times of the year: from winter solstice (Group 1, n = 14) with increasing daylengths (from 8 to 16 h) and from summer solstice (Group 2, n = 8) with decreasing daylengths (from 16 to 8 h). The light regimens were then reversed for Groups 1 and 2. After the first 6-month cycle, the animals in Group 1 were allocated to two groups and maintained under the same initial light regimen but submitted to two ambient temperatures: Group 1 (n = 7) was maintained at 20 ± 2°C and Group 3 (n = 7) was transferred to a cold environment (5 ± 1°C). In control and experimental animals, testicular volume was estimated and blood samples were obtained twice a month to measure plasma testosterone and LH concentrations by radioimmunoassay. In all groups, all the parameters of the reproductive activity studied (testicular volume, testosterone and LH concentrations) were entrained and synchronized by the 6-month light rhythm and two cycles were observed in a year. Reproductive activity was maximum during the long days (light > 12 h) and minimum during the short days (light < 12 h). In the experimental animals and in the controls, the amplitude of variations in the parameters studied were similar. The recrudescence of reproductive activity took place just before the artificial spring equinox (short and increasing daylengths), whereas regression always occurred near the autumn equinox (12 h light:12 h dark), as in the controls kept in a natural environment. The regular incidence of involution at the autumn equinox indicates that there is a period of photosensitivity to decreasing daylengths in summer. In the experimental animals, the resting season was usually 2 months. A comparison of Groups 1 and 2 that had undergone reversed light regimens also showed that the reproductive parameters were driven in opposition. In Groups 1 and 3, no significant effect of ambient temperature (high or low) on the entrainment of the reproductive cycle by the photoperiodic rhythm was observed. These results clearly indicate that photoperiod is of prime importance among the environmental factors controlling reproduction in hedgehogs and can entrain and synchronize the seasonal changes of the reproductive cycle.

Introduction

In natural conditions, mammals from temperate and arctic regions regulate and adjust their reproductive cycle to a period of one year according to external factors. Among these external factors, seasonal changes in daylength are constant from year to year, unlike other environmental factors such as temperature, rainfall and food availability. Daylength is, therefore, a good predictor of the time of the year, which it is necessary to anticipate for the timing of breeding (Turek and Campbell, 1979; Lincoln and Short, 1980; Ortavant et al., 1985). The role of photoperiod in the regulation of reproduction was first established on animals subjected to transequatorial displacement (Marshall, 1937; Thwaites, 1965), or submitted to reversed annual photoperiodic schedules (Wodzicka-Tomaszewksa et al., 1967; Pelletier and Ortavant, 1970; Karsch et al., 1984). More recently accelerated photoperiodic rhythms have been used, with different patterns of light changes, including sine variations (Rougeot, 1969; Lindsay et al., 1984; Pelletier et al., 1986; Pohl, 1987), alternating periods of long and short days (Lincoln and Peet, 1977; Lincoln, 1978) and more complex designs, with
light provided in two daily photofractions (Pelletier and Thimonier, 1987).

In hedgehogs, which are seasonal breeders, the cycle of reproduction in the natural environment is characterized by a resting period in autumn and a period of maximum activity from February–March to August–September (Saboureau and Boissin, 1978; Saboureau and Dutourné, 1981). In adult male hedgehogs, the seasonal variations in gonadal and pituitary activities are parallel during the year (Fowler, 1988; El Omari et al., 1989). The reactivation of reproduction occurs from the beginning of winter (low and increasing daylengths) to a period of low temperatures, and the involution is regular at the end of winter when daylengths are decreasing and temperatures are still high (Saboureau and Boissin, 1983). In hibernating mammals, the regular alternation of reproduction and hibernation is often related to endogenous rhythms (Boissin and Canguilhem, 1988), as exemplified by the entry in hibernation at the beginning of autumn and the spontaneous reactivation of reproduction before the end of hibernation. There have been numerous studies on the effect of temperature in this species, but research on the effect of light has been limited to some data relative to the temporal relationships of the daily and seasonal locomotor activity rhythms (Saboureau et al., 1979), and to an attempt to explain the regulation of gonadal activity (Saboureau, 1981). Consequently, the exact role of photoperiod on the entrainment and synchronization of the reproductive cycle has not yet been established.

The present study was therefore undertaken to determine whether the reproductive cycle in male hedgehogs was effectively synchronized and driven by photoperiodic changes. For this purpose, changes in the testes (volume and plasma testosterone concentrations), and in gonadotrophin concentrations (plasma LH concentrations) were studied in animals submitted to artificial light regimens lasting 6 months and uniform temperature conditions. These were compared with control animals kept under natural climatic conditions.

Materials and Methods

Animals

Male hedgehogs (Erinaceus europaeus) used in this study were born in the Centre d'Études Biologiques de Chizé in southwestern France (46°07'N, 0°25'W). The animals were housed individually and kept under natural climatic conditions of light, temperature and rainfall until adulthood, at the beginning of the experiments. They were fed daily with a mixture of crushed chicken meat and dog biscuits (Canina: Duquesne-Purina, St-Quentin); water was always available.

Experimental design

Animals were allocated randomly to control, or treatment groups. Six animals (controls) were kept outdoors under natural variations of daylight and environmental temperature during the whole time of the experiments. Groups of experimental animals, kept in light-proof rooms, were submitted, at two times of the year (from winter and summer solstices) to an accelerated (six months) artificial light regimen and uniform ambient temperature conditions. The rooms were illuminated by fluorescent tubes simulating the full visible and ultraviolet spectrum of natural white light. The light intensity was about 100 lux at the level of the animal; the regimen was varied to fit a sine curve and was manually adjusted every two days. The amplitude of daylength varied from a minimum of 8 h to a maximum of 16 h; the onset and end of lighting was controlled by an electronic clock.

On the day of the winter solstice, 14 animals were transferred from outdoors (8 h light) to the 6-month artificial light regimen with daylength increasing from 8 to 16 h over the six month period; the ambient temperature was maintained high and constant (20 ± 2°C) during the 18 months of the experiment (Group 1).

On the day of the summer solstice, eight animals were transferred from outdoors (16 h light) to a similar 6-month artificial light regimen with daylength decreasing from 16 to 8 h over the six month period: the ambient temperature was maintained at 20 ± 2°C during the 12 months of the experiment (Group 2).

Groups 1 and 2, therefore, both had 6-month light regimens, but in opposite phases.

After the first 6-month cycle the animals in Group 1 were allocated to two groups and maintained under the same initial light regimen but submitted to two ambient temperatures: Group 1 (n = 7) was maintained at 20 ± 2°C and Group 3 (n = 7) was transferred to a cold environment (5 ± 1°C).

Collection of blood samples

Every two weeks, at the same time in the morning (3–5 h after dawn), a single blood sample was taken, from control and experimental animals, by intracardiac puncture under light halothane anaesthesia (Fluothan: Pitman-Moore France S. A., Meaux). Blood samples were placed into heparinized tubes and were kept on ice until centrifugation (3900 g, 10 min). Plasma was divided into several aliquots and stored at −25°C until used in assays.

At the same time, the body mass of the animals was recorded and the testicular volume was estimated by palpation according to an arbitrary unit scale (from 1 to 5 a.u.); this index correlated with measured data (Saboureau, 1981). Testes are in an intra-abdominal position in hedgehogs and this procedure avoids injury resulting from laparotomy.

Hormone assays

Testosterone assay. Plasma testosterone concentrations were measured by a radioimmunoassay method without chromatography (Saboureau and Dutourné, 1981). Intra- and interassay coefficients of variation were 4 and 10%, respectively, and the sensitivity was <10 pg per tube.

LH assay. Plasma LH was determined by a double-antibody heterologous radioimmunoassay as described by El Omari et al. (1989). Purified rabbit LH (RbLH, AFP-559; NIAMDD, NIH: Bethesda, MD) was used as standard and, also, for radioiodination. An anti-rat LH serum (used at a final dilution of 1:65 000) and an anti-rabbit γ-globulin serum raised from sheep (INRA, Nouzilly) were used as first and precipitating second antibodies, respectively. The sensitivity of the method, determined as the minimal detectable standard dose, was less than 7.9 pg per assay tube, i.e., less than 0.20 ng ml⁻¹ plasma. Serial
and LH concentrations increased in two main phases. The first phase occurred from January–February to April, with the highest plasma concentrations (testosterone 14–17 ng ml⁻¹; LH > 0.6 ng ml⁻¹) and the second from June to August with lower, intermediate concentrations (testosterone 5–9 ng ml⁻¹; LH > 0.5 ng ml⁻¹). Reproductive regression occurred from mid-August to mid-September, during long, but decreasing daylengths (13.5–12 h), in spite of high ambient temperatures. In autumn, during the resting season, all the parameters were minimum (testicular volume < 1.5 a.u.; testosterone 0.2–1.5 ng ml⁻¹; LH 0.3–0.4 ng ml⁻¹). Recrudescence of reproductive activity began spontaneously in December–January, while the animals were still hibernating, and even though daylengths were at their shortest (8–9 h) and ambient temperatures at their lowest for the year.

**Effect of a 6-month artificial light regimen beginning in December (winter solstice) with daylength increasing (from 8 h to 16 h) (Group 1)**

From the winter solstice, male hedgehogs with low testicular activity (testicular volume < 2.0 a.u.; testosterone 5.96 ± 0.84 ng ml⁻¹) but already high LH concentrations (0.51 ± 0.07 ng ml⁻¹) were transferred from their natural environment to the artificial 6-month light regimen and maintained at a constant high ambient temperature (20 ± 2°C). Under these conditions, all the parameters of reproductive activity (Fig. 2) correlated highly and positively with the duration of the light phase, as they were entrained and synchronized by the 6-month light rhythm. During the 18 months of the study, three reproductive cycles were observed. Testicular volume and plasma concentrations of testosterone and LH were at a maximum during long days (light > 12 h), at a minimum during short days (light < 12 h), and showed the same amplitude of variation as in the controls. During the period of maximum activity, the patterns of testosterone and LH were characterized by a high peak (testosterone ≈ 20 ng ml⁻¹ on days 30, 240 and 435; LH ≈ 0.6–0.7 ng ml⁻¹ on days 75, 255 and 435) during the increasing long days (light from 12 h to 16 h) and a significant decrease (P < 0.05) to a lower plateau value (testosterone 10–15 ng ml⁻¹; LH ≈ 0.4 ng ml⁻¹) after the artificial summer solstice (light from 16 h to 12 h). The recrudescence of reproductive activity took place as the short days increased in length (light from 10 h to 12 h), just before the spring equinox for testosterone and LH, and 15 days before for testicular volume. Regression was also rapid and significant for testosterone (P < 0.01) and LH (P < 0.05) after the autumn equinox (light from 12 h to 10 h) with the decrease in testicular volume occurring 15 days later. The resting period, with the lowest concentrations of testosterone (0.2–0.6 ng ml⁻¹) and LH (<0.3 ng ml⁻¹), had a duration of about two months. As in control animals, involution always occurred after the autumnal equinox (light < 12 h).

**Effect of a 6-month artificial light regimen beginning in June (summer solstice) with daylength decreasing from 16 to 8 h (Group 2)**

At the beginning of the second light regimen, which occurred during the second part of the reproductive season, testicular
Fig. 2. Effects of a 6-month artificial light regimen (top of figure) beginning in December (winter solstice) with increasing daylengths and a high constant ambient temperature (20 ± 2°C) on (a) the testicular volume (•) and plasma testosterone concentrations (●) and (b) LH concentrations in male hedgehogs (n = 14 from days 0–180 and n = 7 afterwards). Data are means ± SEM.

Fig. 3. Effects of a 6-month artificial light regimen (top of figure) beginning in June (↓ = summer solstice) with decreasing daylengths and under a high constant ambient temperature (20 ± 2°C) on (a) the testicular volume (●) and plasma testosterone concentrations (●) and (b) LH concentrations in hedgehogs (n = 8). Data are means ± SEM. NP: natural photoperiod.

Effect of two ambient temperatures (high: 20 ± 2°C versus low: 5 ± 1°C) with the same 6-month artificial light regimen (Group 3)

In Group 3, the effect of a low ambient temperature (5 ± 1°C) with the same initial light regimen was studied after the animals had been exposed to a first 6-month cycle at high ambient temperature (20 ± 2°C). At 5°C, testicular volume and testosterone and LH patterns were also entrained to the 6-month light rhythm (Fig. 5) as in the other experiments. Nevertheless, LH concentrations stayed significantly higher at 5°C than at 20°C (< 0.4 ng ml⁻¹ (20°C, days 105–135) versus > 0.6 ng ml⁻¹ (5°C, days 300–315 and days 450–465): P < 0.01) during the second part of the period of reproduction (decreasing long days). Irrespective of ambient temperatures, the involution of reproductive activity always occurred soon after the autumn equinox.

The superimposition of data from Groups 1 and 3, as illustrated by the profiles of testosterone concentrations (Fig. 6),
confirmed that the effect of different ambient temperatures is of little importance in the entrainment of gonadal activity in male hedgehogs, compared with the effect of the photoperiodic regimen. However, a comparison of the patterns of testosterone of Groups 1 and 3 indicated that at 5°C, there had been a 15 day advance in (a) the regression phase (end of the first cycle) at the autumn equinox (on day 315 the testosterone concentration for Group 1 was 11.08 ± 1.75 ng ml⁻¹ compared with 4.16 ± 1.50 ng ml⁻¹ for Group 3; P < 0.05) and (b) the following recrudescence phase (beginning of the second cycle) at the spring equinox (on day 405 the testosterone concentration was 4.03 ± 0.97 ng ml⁻¹ for Group 1 compared with 16.59 ± 6.15 ng ml⁻¹ for Group 3; P < 0.05).

Discussion

These results show, for the first time in male hedgehogs, that reproductive activity (testicular volume, plasma testosterone and LH concentrations), observed in the natural environment (El Omari et al., 1989), can be entrained by an artificial light regimen. The results also demonstrate that the artificial light regimen induced significant and reproducible changes in gonadal activity. In our experiments, hedgehogs submitted to an accelerated artificial light regimen that mimics in six months the photoperiodic changes that normally occur in twelve months, presented two successive reproductive cycles in one year, while the controls had only one. The characteristic changes in pituitary–gonadal activity that occurred annually were also observed under these accelerated light regimens and followed exactly the variations in daylength (i.e. maximum endocrine activity during long days (light > 12 h) and minimum activity during short days (light < 12 h)). Two reproductive cycles a year have previously been observed in males and females of many domestic and wild mammals when submitted to similar semestrial photoperiodic rhythms (Rougeot, 1969; Lindsay et al., 1984; Pelletier et al., 1986; Pohl, 1987).

Acceleration of the photoperiodic cycle resulted from either the winter solstice, with increasing daylengths (Group 1), or the summer solstice, with decreasing daylengths (Group 2). The animals responded to daylength and pituitary–gonadal activity was adjusted accordingly. During the first artificial cycle (Group 1), the fact that the maximum activity period was longer than in the second and third cycles may be related to acclimatization to phototreatment as reproductive activity was already raised at the beginning of the experiment. In Group 2, however, the...
acceleration of the decrease of daylength entrained a rapid involution of reproductive activity. In Groups 1 and 2, animals were submitted to reversed 6-month light cycles, under the same environmental parameters (food ad libitum, temperature 20 ± 2°C), and the reproductive changes were driven in exact opposition. These results demonstrate the strength of photoperiod as an entraining agent of the reproductive activity in hedgehogs, and is in agreement with work on animals subjected to transsequatorial displacement (Marshall, 1937; Thwaites, 1965) or submitted to reversed annual photoperiodic schedules (Wodzicka-Tomaszewkska et al., 1967; Pelletier and Ortavant, 1970; Travis and Pilbeam, 1980; Karsch et al., 1984).

Exposure to a cold environment (5°C) (Group 3) did not prevent the entrainment of testicular volume and hormone changes by the semestral artificial light cycle, and the pattern of the reproductive cycle was almost similar, in amplitude and in phase, to Group 1 (20°C). These results demonstrate, in hedgehogs, hibernating mammals, that the influence of low temperature, generally considered of prime importance, is subordinate to that of the photoperiod, as is the case in non-hibernating mammals (Wodzicka-Tomaszewksa et al., 1967). Nevertheless, in Group 3, the small advance of the regression, at the artificial autumn equinox, may be related to a sensitivity to low temperatures as already observed in hedgehogs in summer (Saboureau, 1981). In golden hamsters, it has also been shown that the testicular involution induced by short photoperiods was accelerated by low temperatures (Pevet et al., 1986). The similar shift observed during the following recrudescence, before the artificial spring equinox, is more difficult to explain, unless gonadal reactivation is spontaneous and occurs, as observed in all other experiments under semestral light regimen, nearly two months after the beginning of the resting phase. The increase in LH concentrations in animals kept at 5°C during the second part of the activity period cannot be explained at the moment, but it may be due either to a stimulatory effect of the low temperature on pituitary hormone secretion or the consequence of a lower metabolic turnover of LH.

In hedgehogs the reproductive cycle seems to be endogenous (Saboureau, 1981) and is adjusted to natural conditions over the period of one year by external factors, as in many other mammals, in which endogenous reproductive rhythms are reported (sheep: Howles et al., 1982; Karsch et al., 1989; Malpaux et al., 1989; ground-squirrel: Licht et al., 1982; ferret: Boissin-Agasse et al., 1985; Boissin and Canguilhem, 1988; mink: Martinet et al., 1992). The present results show that the photoperiod can be considered, among the external factors, as an important environmental ‘Zeitgeber’ for entrainment of seasonal reproductive function in hedgehogs.

The mechanisms by which light regulates the pituitary–gonadal cycle in hedgehogs, under natural or experimental conditions, can now be specified. Nevertheless, previous results have shown that reproductive activity increases before day length increased, or was long enough (between 8 and 12 h) to be stimulatory, either under natural conditions (Saboureau and Boissin, 1978; Saboureau and Dutourné, 1981) or in very severe experimental conditions (such as fasting at low temperatures, and short photoperiod: Saboureau et al., 1984; Saboureau, 1986; El Omari et al., 1987). However, the involution of the testes always occurred rapidly in hedgehogs blinded or submitted to short daylengths (light < 12 h) after the summer solstice (Saboureau, 1981, 1986). Under the 6-month light regimen, these findings are confirmed by the changes in gonadotrophic and testicular activities, particularly by the regularity of the decrease of reproductive activity at the artificial autumn equinox (12 h light:12 h dark). Consequently, it appears that reproduction in hedgehogs, as in some rodents (Reiter, 1973, 1974; Hoffmann, 1979; Johnston and Zucker, 1980; Boyd, 1986), is actively photoregulated, and is inhibited by the decreasing photoperiods occurring before or near the autumn equinox. As the photoperiodic time measurement is based on the existence of a circadian rhythm of photosensitivity (Elliott, 1976), it may be postulated that such a period of sensitivity to decreasing daylengths, limited to summer and early autumn, occurs in hedgehogs. In autumn and winter, the regular, spontaneous recrudescence of pituitary–testicular activity, occurring after several months of rest, may correspond to the development of refractoriness to short days at the central level. This hypothesis is in accordance with similar results obtained in hamsters (Turek et al., 1975; Turek and Campbell, 1979, 1989; Reiter, 1980; Hoffmann, 1981).

Thus, in hedgehogs, as in many other photoperiodic species, the pineal gland and its hormonal secretions seem to be involved in the photoperiodic regulation of the gonadal cycle (Elliott, 1976; Reiter, 1980, 1983; Arendt et al., 1981; Hoffmann, 1981, 1985; Vivien-Roels and Pevet, 1983). This contention is supported by studies that showed that ultrastructural changes occurred in pinealocytes during the sexual cycle (Pevet and Saboureau, 1973), that exogenous melatonin induced gonadal regression in the summer (Saboureau, 1973), and an advance in the timing of the testicular reactivation in winter (Fowler and
Racey, 1990). Sabourau et al. (1991) have also shown that photoperiodic changes are communicated via projections of the superior cervical ganglia to the pineal gland and are rapidly converted into endocrine messages. In conclusion, in hedgehogs, photoperiod is of prime importance in the control of reproduction and it entrains and synchronizes the endogenous rhythm of reproduction. As in many other photoperiodic species, periods of photosensitivity during the year may be involved, and the pineal gland is implicated in these regulations. Further work is needed to determine how hedgehogs measure photoperiodic time over the annual cycle, and to determine, at the central level, the mechanisms and structures implied in these neuroendocrine regulations.

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