

Photoperiod and parturition period in isolated or paired rats : influence of sight deprivation and social conditions

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Summary — Time of delivery was studied in normal (N) or enucleated (E) rats. Isolated (1/cage) at mating (day 1 of gestation), they were all anaesthetized on day 8 and some of them were then blinded. After anaesthesia, they were kept either alone (N and E) or in homogeneous (NN and EE) and heterogeneous (NE) pairs either under a 12L—12D or a 2L—22D light—dark (LD) cycle. Activity, monitored throughout the rest of pregnancy in 2 homogeneous (NN and EE) and heterogeneous (NE) pairs of rats, kept a marked circadian rhythm under each light regimen. Birth distributions differed according to LD cycle in normal (N or NN) but not in enucleated (E and EE) rats. Under the 12L—12D regimen, parturitions of N and E rats were divided into 2 parts, the majority occurred on the afternoon of day 22 before 21:00 h, the remainder were observed after 6:00 h on day 23. Under the 2L—22D regimen, N rats gave birth over one period, mainly on day 23, whereas E rats had the same birth distribution as those subjected to the 12L—12D LD cycle. In heterogeneous pairs of rats (NE), birth times were affected by photoperiod; under the 2L—22D regimen it was intermediate between those of homogeneous pairs (NN and EE). These results indicate that the eyes were the first link of the nervous chain by which photoperiod influenced birth time. Social conditions may also modulate the photodependent mechanism in ways which remain to be determined.

rat — parturition — photoperiod — eyes — physical activity — social conditions

Résumé — Photopériode et moment de la parturition chez la rate élevée seule ou par deux. Influences de la privation de la vue et des conditions sociales. Le moment de la parturition a été étudié chez des rates normales (N) ou énucléées (E). Isolées après la saillie (jour 1 de la gestation ou J1), elles ont été anesthésiées à J8 et certaines d'entre elles ont été alors énucléées. Après l'anesthésie, elles ont été gardées isolées (N ou E) ou en paires homogènes (NN et EE) et hétérogènes (NE) sous des régimes photopériodiques 12L—12D et 2L—22D. De J9 à la fin de la gestation, l'activité générale, enregistrée chez deux paires homogènes et deux paires hétérogènes, a conservé un rythme circadien sous chaque régime photopériodique. Les distributions des naissances ont été différentes selon le régime «jour—nuit» chez les rates normales (N ou NN) mais non chez les énucléées (E ou EE). Sous le cycle 12L—12D, les parturitions des rates normales et

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énucléées se sont réparties en deux groupes; elles se sont produites en majorité au cours de l'après-midi du 22^e jour de la gestation avant 21:00 h, les autres ont été observées après 6:00 h le 23^e jour. Sous le cycle 2L—22D, les rates normales ont mis bas sur une seule période principalement à J23 alors que les rates énucléées ont présenté une distribution des naissances équivalente à celle des rates soumises au cycle 12L—12D. Chez les paires hétérogènes (NE), les moments des naissances ont été affectés par le régime photopériodique. Sous le cycle 2L—22D, leur distribution a été intermédiaire entre celles des paires homogènes (NN et EE). Ces résultats indiquent que les yeux sont le premier maillon de la chaîne nerveuse par laquelle la photopériode influence le moment de la naissance. Ils montrent aussi que les conditions sociales peuvent affecter ce mécanisme par d'autres voies qui restent à déterminer.

rat — moment de la naissance — photopériode — vue — conditions sociales

Introduction

In rats, photoperiod modulates the time of birth (Mitchell and Yochim, 1970; Plaut *et al.*, 1970; Lincoln and Porter, 1976; Bosc and Nicolle, 1980a), and therefore the birth mechanism, by endogenous events which are finally integrated at the uterine and cervical levels. Other environmental cues can also modify the time of birth in this species, as in the case of periodic food presentation (Bosc and Nicolle, 1985; Bosc *et al.*, 1986) and probably of social environment (Bosc and Nicolle, 1985). In order to evaluate the possibility of a social influence on the time of parturition, pairs of normal and/or blinded rats were subjected to two different light regimens. The two types of animals retained for this study also offered an opportunity to determine if the eyes were the first link in the neural pathway which mediates the effects of light on birth time.

Materials and Methods

Animals

Rats from strain 03 of our colony (Wistar origin) were kept in constant-temperature rooms (20—21°C) under a standard light regimen of 14 h of light and 10 h of darkness (14L—10D) with lights on from 06:00 to 20:00 h (local

winter time). After the age of 3 months, females were caged with a male for a night (a mating session). Mating (day 1 of pregnancy) was assessed by the presence of spermatozoa in the vaginal smear. Mated females were isolated one per cage and allocated to one of the experimental groups. The number of rats used in each group ranged between 20 and 44 for a total number of 325. Food (commercial pellets, U.A.R., Paris) and water were available *ad libitum* and renewed 2 or 3 times a week. The litter and cage were changed twice during gestation, the former on day 8 and the latter about one week later. Several mating sessions were necessary to complete all groups except one which was constituted in one night.

Experimental groups

The protocol included the anatomical status, the number of rats per box and the photoperiod. The anatomical status referred to two types of animals, normal (N) and enucleated (E). All rats were anaesthetized on day 8 of gestation with diethyl oxide; they were weighed and the eyeballs of the enucleated rats were removed. Just after anaesthesia and/or after surgery, the rats were caged alone or in pairs to constitute the following groups: N (one normal), E (one enucleated), NN (two normals), EE (two enucleated) and NE (one normal + one enucleated). Under a 12L—12D regimen (lights on from 06:00 to 18:00 h) a total of 102 parturitions were observed in these groups which were called: N₁₂ (*n* = 19), E₁₂ (*n* = 18), NN₁₂ (*n* = 21), EE₁₂ (*n* = 25) and NE₁₂ (*n* = 19). Under a 2L—22D regimen (lights on from 06:00 to 08:00 h), 91 parturitions were recorded and the groups were then named: N₂ (*n* = 20), E₂ (*n* = 20), NN₂ (*n* = 19), EE₂ (*n* = 14) and NE₂ (*n* = 18). The two light

regimens (12L—12D and 2L—22D) were started on day 9 of gestation and continued up until the end of the last parturition. At each mating session, several groups were put together in a room with a special light regimen. An additional group of normal rats (one per cage) was constituted after one mating session and subjected to continuous dim light (group LL, $n = 19$) from day 9 of gestation. This regimen was achieved as previously reported (Bosc, 1987), by placing an opaque shade around the light source in such a way as to indirectly illuminate the animals; at their levels, 0.6—1.4 lux were obtained (Luxmètre CL2010; Chauvin & Arnoux, Paris).

Observations and analyses

The rats were inspected every hour from day 22—10:00 h, and the hour preceding the observations of the first young was considered as the time of birth. Litters with more than 2 young were retained for analysis and cumulated birth rates were then plotted and compared according to the non-parametric test of Kolmogorov—Smirnov (Siegel, 1956). An interval between birth and a given reference point (day 22—00:00 h) was also taken into account to compare the timings of parturition (variance analysis) (Vessereau, 1960).

The homogeneity of the groups was assessed on the basis of litter size, stillbirth rates and birth weight of live young (taken about half a day after parturition). Fertility was also compared among groups by taking into account all pregnant females (chi-square analysis) (Vessereau, 1960). Weights of the dams on day 8 of gestation and just after parturition were used as indicators of adaptation after surgery.

Activity was monitored from day 9 of gestation until parturition, as previously described (Bosc *et al.*, 1986) in two pairs of rats in groups NN₁₂, NE₁₂, EE₁₂, NN₂, NE₂ and EE₂. The main times of activity for each cage were visualized from day 10 of pregnancy until parturition. The hours during which the rats were active for at least 25% of the time were determined and represented by a dark horizontal bar for each 24 h period, each day being represented by a line underneath the bar. These records showed a pattern of activity resulting from the treatments. Subsequent analysis comprised the hourly times of activity over 12 consecutive days. The autocorrelations (Chatfield, 1978) were computed. In addition,

periodogram analysis was performed with test periods varying between 12 and 30 h at 0.5-h increments, according to the procedure of Enright (1965) adapted by Sokolove and Bushell (1978). All calculations were made on a Bull computer with appropriate programs (Becker and Chambers, 1984; David, 1984).

Results

The first parturitions were observed around noon of day 22 of gestation in all groups. Those following were spread over one or two periods, according to the group and to the light regimen.

When rats were kept alone, there were two periods of birth for groups N₁₂ and E₁₂ under the 12L—12D regimen and for group E₂ under the 2L—22D one. In the first period, births occurred mainly between 12:00 h and 21:00 h on day 22. In groups N₁₂, E₁₂ and E₂ ($P > 0.05$), 63.1, 52.9 and 66.7% of the births were then respectively observed before 24:00 h; the remaining ones occurred on day 23 during the second period starting at about 6:00 h, and the following 7 and 9 h without deliveries corresponded to the night for group N₁₂ (Fig. 1). By contrast, group N₂ rats under the 2L—22D regimen gave birth over one period; 26.3% of deliveries were seen on day 22 and 73.7% on day 23. This group differed from the preceding ones ($P < 0.05$).

When rats were grouped in pairs, births were also divided into two periods under the 12L—12D regimen for groups NN₁₂ and NE₁₂. In these groups, 70.0 and 78.9% of births were observed on day 22, a slightly higher percentage than in groups N₁₂ and E₁₂ ($P > 0.05$) over the same times. The remaining ones occurred 7 and 9 h later after 6:00 h on day 23. By comparison, births occurred in one period for groups NN₂ and NE₂ under the

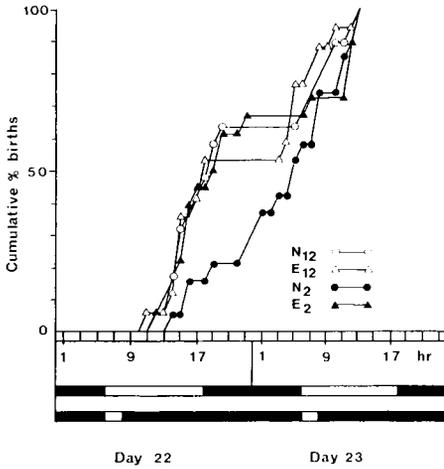


Fig. 1. Birth distribution on days 22 and 23 of gestation in normal (N) or blinded (E) rats caged alone under two light regimens. Groups N_{12} (○—○; $n = 19$) and E_{12} (△—△, $n = 17$) were under a 12L—12D regimen. Groups N_2 (●—●, $n = 19$) and E_2 (▲—▲, $n = 18$) were under a 2L—22D regimen. The two light regimens are indicated at the bottom. Group N_2 is also represented in Fig. 2.

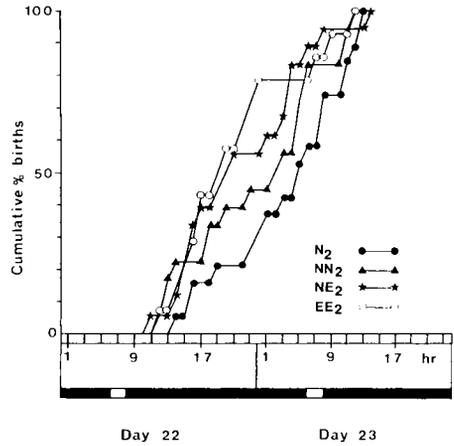


Fig. 2. Birth distribution on days 22 and 23 of gestation in normal (N) or blinded (E) rats caged alone or by twos under a 2L—22D regimen. Rats of groups N_2 (●—●, $n = 19$) were caged alone; rats of groups NN_2 (▲—▲, $n = 18$), NE_2 (*—*, $n = 18$) and EE_2 (○—○; $n = 14$) by two. The light regimen is indicated at the bottom. Group N_2 is also represented in Fig. 1, group NE_2 in Fig. 3 and group EE_2 in Fig. 4.

2L—22D regimen; 44.4 and 55.6% were, respectively, observed before midnight on day 22, the others occurring later. Fig. 2, showing these groups together with groups N_2 and EE_2 , illustrates the effects of anatomical status and of the number of rats per cage. When the interval between day 22—00:00 h and birth was taken into account, the mean times of parturition were, respectively : 27:46 h (SD = 7:25 h), 24:36 h (SD = 7:59 h), 21:53 h (SD = 7:30 h) and 21:21 h (SD = 7:23 h) for groups N_2 , NN_2 , NE_2 and EE_2 ($P < 0.05$ for N_2 vs NE_2 and EE_2).

If the difference due to the light regimen was obvious for normal rats kept alone or in homogeneous pairs (groups N_{12} and NN_{12} vs groups N_2 and NN_2), (Fig. 1), it was clear also for the heterogeneous pairs (groups NE_{12} and

NE_2) (Fig. 3) (median test : $0.05 < P < 0.1$). However, in no group formed by these pairs, did birth times differ between the types of rat ($P > 0.05$). Finally, rats alone under continuous light (group LL) gave birth mainly on day 22 (77.8%) as did rats of groups EE_{12} and EE_2 (63.6 and 71.4%, respectively; $P > 0.05$) (Fig. 4).

Activity was concentrated within defined daily hours which were limited to the night in group NN_{12} (Fig. 5) or to the hours corresponding to the night of their preceding light regimen in group NN_2 (Fig. 5). Blinded rats of groups EE_{12} (Fig. 5) or EE_2 (Fig. 5) also evidenced main daily times of activity during the hours corresponding approximately to the night of their regimen preceding surgery. In pairs comprising normal and blinded rats, the same patterns were also observed

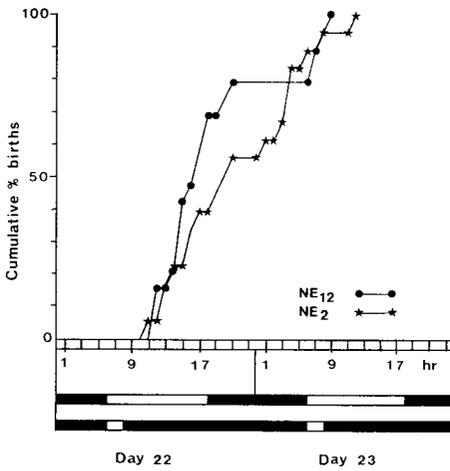


Fig. 3. Birth distribution on days 22 and 23 of gestation in pairs comprising normal and blinded rats under two light regimens. Rats of groups NE_{12} (●—●, $n = 19$) were under a 12L—12D regimen. Rats of group NE_2 (*—*, $n = 18$) were under a 2L—22 D regimen. The two light regimens are seen at the bottom. Group NE_2 is also represented in Fig. 2.

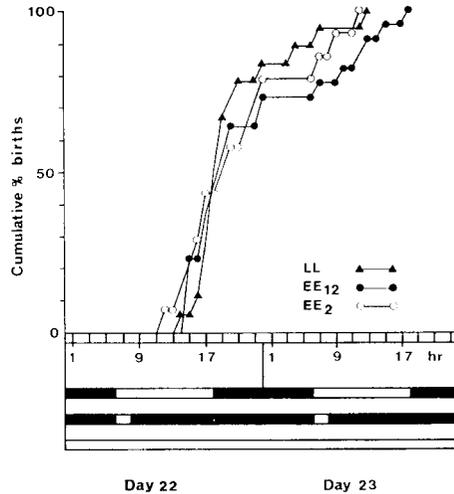


Fig. 4. Birth distribution on days 22 and 23 of gestation of normal rats caged alone or in pairs of enucleated (E) rats under different light regimens. Normal rats of group LL (▲—▲, $n = 18$) were under a dim constant light. Rats of groups EE_{12} (●—●, $n = 22$) and EE_2 (○—○, $n = 14$) were under a 12L—12D and a 2L—22D regimen, respectively. The light treatments are represented at the bottom. Group EE_2 is also represented in Fig. 2.

(Fig. 5 for group NE_{12} , Fig. 5 for group NE_2); in a few cases, the daily activity was slightly shifted. The homogeneous pairs (groups NN_{12} , NN_2 , EE_{12} and EE_2) had autocorrelations which followed a clear sinusoidal pattern with an apparent period of 24 h. This circadian periodicity was confirmed by the periodogram estimates (Fig. 6). The same period was also found in the heterogeneous pairs (groups NE_{12} and NE_2) (Fig. 6) but the autocorrelations were less regular than in the other groups indicating some disturbances of circadian rhythm of activity.

All groups were homogeneous for their litter size (9.9 ± 2.5) and the number of live pups at birth (9.6 ± 2.4). There were however some variations, group E_{12}

having the smallest litter size (8.6 ± 3.3) and one of the largest stillborn rates (8.9%). Stillbirth rates ranged between 2.6 and 13.7% and were not associated with particular treatments. Birth weights of live young did not differ among the groups ($P > 0.05$). However, fertility was affected by anatomical status ($P < 0.001$), extirpation of the eyes leading to a significant reduction (54.7 vs 76.8%). Fertility was particularly low in groups E_2 (45.4%) and EE_2 (43.7%). The dams were on the average a little heavier just after parturition than on day 8 of gestation in all groups, except in group E_2 in which they were a little lighter (on the average 7 g less).

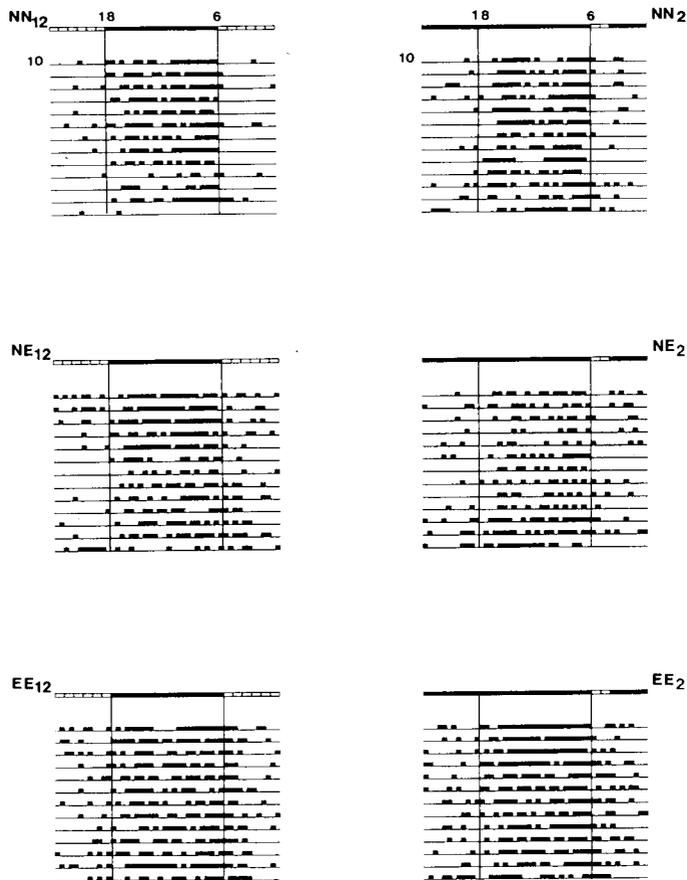


Fig. 5. Main daily periods of activity during the last 13 days of gestation in pairs of normal (N) or enucleated (E) rats. The light regimens (12L—12D or 2L—22D) are given at the top of each panel (a pair of rats). Each line represents a 24-h period starting on day 10 of pregnancy at 12:00 h. Horizontal dark bars indicate an activity time of > 25% of the corresponding hour. Vertical lines indicate the dark phase of the 12L—12D regimen.

Discussion

A comparison between groups of normal and blinded rats indicates that the eyes were the first link in the nervous pathway by which photoperiod influenced birth time in the rats. This agrees with the general assertion that light entrainment of

circadian rhythms is mediated by the retina in mammals. Rodents do not have extra-retinal light perception (Moore, 1979; Rusak and Zucker, 1979), even if light is directly transmitted to the brain as shown in adult ewes (Van Brunt *et al.*, 1964) or in rats and guinea pig fetuses (Jacques *et al.*, 1987).

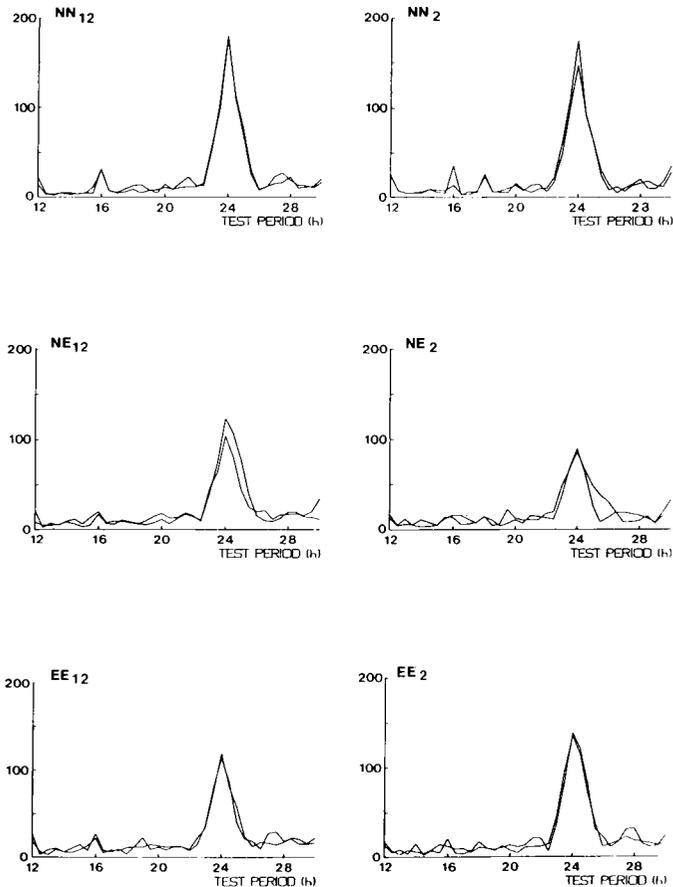


Fig. 6. Periodogram estimates of the hourly physical activity in pairs of normal (N) or enucleated (E) rats. Under each light regimen, two pairs of rats have been recorded. Groups NN₁₂, NE₁₂ and EE₁₂ were under a 12L—12D regimen. Groups NN₂, NE₂ and EE₂ were under a 2L—22D regimen.

This experiment also shows that social environment may influence the time of parturition in rats. The procedure of housing rats alone or by twos created social conditions which gave different birth distributions according to the anatomical status of the animals and the light regimen. Thus a pair comprising one normal and one enucleated rat gave a response intermediate between the homogeneous pairs of normal or

enucleated animals under the 2L—22D regimen but not under the 12L—12D (Figs. 2 and 3). This may be attributed to a reciprocal influence resulting from the effect of photoperiod on normal rats and from its lack of effect on the enucleated ones. This reciprocal influence therefore indicates the existence of a mechanism of substitution, the perception of light by the eyes being replaced by another stimulus which may act on the regulation of

parturition. Birth is not the only reproductive event which may be affected by social control and this is not limited to the rat (McClintock, 1981). The problem now is to determine by which mechanism this environmental cue regulates birth time. In this respect, it would be interesting to know if this mechanism implicates the suprachiasmatic nuclei which mediate the effect of photoperiod on birth timing (Murakami *et al.*, 1987; Reppert *et al.*, 1987). It should be noted here that the social conditions were limited to the close environment of the animals but not to the general context, *i.e.*, the room under the present conditions. Groups N₂, NN₂, NE₂ and EE₂ were in the same room (or light regimen) but had different birth distributions (Fig. 2).

This experiment also provides an insight into the endogenous organization of the photodependent mechanism. The pairs of normal rats had the same circadian periodicity of activity under the two light regimens (Figs. 5 and 6). The different distributions of births observed under these conditions (Fig. 1) illustrate therefore the specific effect of light given at a determined moment of the day. Light reduced to two morning hours delayed most of the parturitions to day 23 of gestation, just as a light flash given at the same times under a 8L—16D regimen (Bosc and Nicolle, 1980b). These results confirm that the process of birth depends upon an endogenous rhythm of photosensitivity (Bosc and Nicolle, 1980b). The blinded rats which would free-run after surgery, presented the same circadian rhythm of activity (Figs. 5 and 6) as the normal rats. The apparent stability of this rhythm may be attributed to the short time interval between surgery and the end of gestation and/or perhaps because the rats were caged by twos. One may note also that blinded rats

presented a break in delivery for several hours corresponding to the most active period of the day, as previously observed in other conditions (Bosc *et al.*, 1986). This lack of birth for a significant time indicates that parturition can be initiated during given hours of the day according to a gate system which can be reset by photoperiod or other environmental cues (Bosc and Nicolle, 1985; Bosc *et al.*, 1986).

Variations in litter size or stillbirth rate usually related to length of gestation or parturition cannot explain the differences observed among groups. Some problems of adaptation after surgery were nevertheless encountered in a group of enucleated rats (group E₂). Another effect observed in this experiment is the lower fertility of blinded rats. This decrease of fertility is perhaps due to the stress of surgery after the completion of implantation (Psychoyos, 1973), or it may result from unspecified effects of sight deprivation.

In conclusion, the integration of lighting conditions by the eyes is the way by which photoperiod modulates the time of birth in the rat. The use of normal and blinded animals shows that social environment may also play a role. It is interesting here to point out the flexibility of the neurohormonal mechanism by which birth time may be controlled by environmental cues.

Acknowledgments

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