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Effects of Neonatal Pinealectomy on Postnatal Development of Reproductive Function in Rats

With 1 Text-figure

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ABSTRACT The neonatally pinealectomized rats maintained the estrous cycle, though somewhat irregular, under continuous illumination as did the sham-operated rats. Under cyclic illumination (12 hrs light; 12 hrs darkness), both the sham-operated and the pinealectomized female rats showed 4-day estrous cycle and the cycle was continued throughout the experimental periods. No differences were found between the control and the experimental groups in any index on sexual activities; the age of vaginal opening, the estrous rate and the weight of the sexual organs. Also in males, any significant difference was not found in weight of the reproductive organs between the groups. The pineal gland had no influence to the induction of persistent estrus of rats neonatally exposed to continuous illumination, and to the establishment and the maintenance of the regular cycle in rats under dark-light environment. Effects of the pineal on the postnatal development of the reproductive function is a minor one in rats.

INTRODUCTION

It is well established that young rats placed under continuous illumination take a longer postpuberal period to reach the persistent estrous state (Maekawa, 1959; Everett, 1961; Takahashi and Suzuki, 1969; Daane and Parlow, 1971; Hoffmann, 1973). It has been repeatedly ascertained in our laboratory that the female rats brought up from the neonatal day under continuous illumination maintained the estrous cycle for long time, though the animals kept in the same environment beginning from later or postpuberal age invariably revealed persistent estrus soon after the exposure to continuous illumination (Takeo *et al.*, 1974, 1975 b).

On the other hand, it is well known that the pineal gland remains undeveloped functionally or morphologically in the neonatal rats, though the gland in an adult animal is highly responsive to dark and light environment. Recently, the ontogenetic studies of the pineal body showed a brilliant advance. Serotonin is barely detectable in the pineal glands of newborn rats; its presence is clearly detected in the

3-day-old rats, and the circadian rhythm of serotonin is established as early as 6 days of age (Machado *et al.*, 1969; Illnerová, 1971). Pineal serotonin N-acetyltransferase activity is detectable in the rats as early as 4 days prior to birth and its circadian rhythm in the enzyme activity appears at 4 days of age, and then it develops most rapidly during the second week and achieves an adult magnitude by the end of the third week (Ellison *et al.*, 1972). On hydroxyindole-O-methyltransferase that has the ability to convert N-acetylserotonin to melatonin, no significant activity is found at 10 days or earlier; its activity/gland attains about 40% of an adult value at 14 days and an adult value at a time closely corresponding to puberty (Zweig and Snyder, 1968; Klein and Lines, 1969). According to the fluorescent histochemistry on the development of sympathetic nerve of the pineals, it takes two to three weeks to be completely innervated (Hakanson, 1967; Machado *et al.*, 1968 a, b).

The present experiment was undertaken to study whether the development of the pineal body in newborn rats has any role on the induction of persistent estrus of rats neonatally exposed to continuous illumination.

MATERIALS AND METHODS

Wistar rats bred in our laboratory were used. They were provided with laboratory chow and water *ad lib*. All rats were brought up in transparent plastic cages laying wooden chips before weaning and housed in stainless steel wire cages afterwards. Environmental conditions were controlled with respect to room temperature (20–25°C), humidity (50–60%) and intensity of illumination (500–600 lux).

The 45 female pups were divided into 2 groups of 21 and 24 animals. The 21 rats in group 1 were housed under cyclic illumination (light, 6: 00 to 18: 00; darkness, 18: 00 to 6: 00) from birth to autopsy. The 24 rats in group 2 were placed under continuous illumination from birth to autopsy. The litter-males also were divided into 2 groups and subjected to the same treatment as the females; the 15 rats of 34 males were placed under cyclic illumination (group 3) and the rest were exposed to continuous illumination (group 4). About a half of rats in each group was subjected to pinealectomy and the rest to sham-operation on the next day of birth. All rats were autopsied at 100 days of age.

Vaginal smears were taken every day at approximately the same time (9: 30–11: 30 A. M.) beginning from the day of vaginal opening to autopsy. Operation was performed under cold anesthesia. At first, a sharp knife cut was made transversely to the interparietal bone and the brain-membranes to the extent of about 5 mm length, and then pinealectomy was performed by the rear approach with a pair of sharp forceps. At autopsy, the cranium was opened and checked macroscopically for completeness of pinealectomy. The gonads and the accessory reproductive organs were removed and weighed. Of those, only the ovaries and the testes were fixed in Bouin's solution and embedded in paraffin for microscopic preparation, and 10 μ sections were stained with hematoxylin-eosin.

Results

Vaginal smear records of all groups are shown in Fig. 1. For the expression of vaginal smear records, the method of Maekawa (1956) was used; the thin baseline of

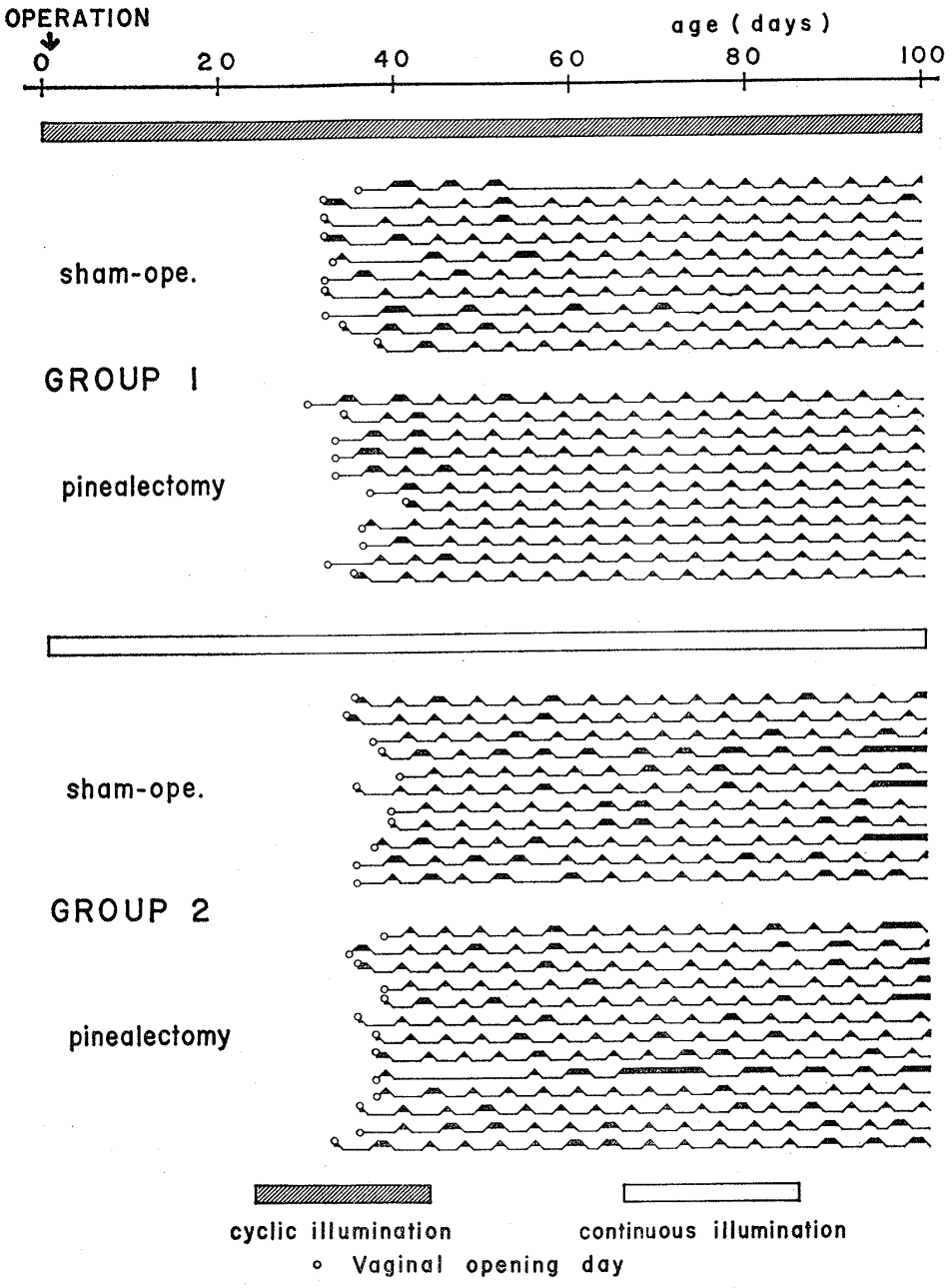

Fig. 1. Vaginal smear records in all female rats in this study. Group 1 was housed under cyclic illumination (12 hrs light; 12 hrs darkness) from birth. Group 2 was placed under continuous illumination from birth. All rats were subjected to sham-operation or pinealectomy at the next day of birth.

the figures corresponds to non-estrous stages which contain proestrus, metestrus and diestrus, and the thick top to perfect estrus.

In group 1, the sham-operated and pinealectomized rats which were housed from birth under cyclic illumination had normal 4-day estrous cycle shortly after the irregular periods. The establishment of the normal estrous cycle was earlier in the pinealectomized group than in the control. The age in days when rats initiated successive five 4-day cycles was defined as the "day of establishment of the normal cyclicity", which was 54.5±3.4 days in the sham-operated group and 45.5±1.5 days in the pinealectomized group. Difference between the two was significant ($P<0.05$). A ratio of the days of perfect estrus to the all days from vaginal opening to autopsy, however, was the same, 26.0% in both subgroups of group 1. Age at the vaginal opening was 33.3±0.7 days in the control and 34.5±0.9 days in the experimental rats.

Table 1
Body weights and organ weights per 100 g body weight in female rats of groups 1 and 2.

Groups	No. of rats	Body weight	Ovaries	Uteri	Adrenals	Hypophysis
		mean ± S. E. (g)	mean ± S. E. (mg)			
(Group 1)						
Sham-operation	10	264.0±6.5	30.4±1.3	192.0±10.6	30.9±1.6	3.7±0.3
Pinealectomy	11	249.0±5.0	27.8±1.0	195.3±15.5	28.6±1.3	3.7±0.2
(Group 2)						
Sham-operation	11	256.8±8.2	28.2±1.6	198.9± 8.2	23.2±0.7	2.9±0.2
Pinealectomy	13	259.8±5.4	28.3±1.0	186.6±10.8	25.1±1.0	3.2±0.2

In group 2, both the sham-operated and pinealectomized rats which were placed from birth under continuous illumination had somewhat irregular cycles which partially contained prolonged estrus, but showed generally 4-day estrous cycles. A ratio, in group 2, of the exactly 4-day cycles to all estrous cycles throughout all experimental periods was 72.9% in the sham-operated and 63.7% in the pinealectomized group. The difference was not significant ($P>0.05$). The incidence of perfect estrus was 31.8% in the control and 31.2% in the experimental rats. Age at the vaginal opening was 36.7±0.6 days in the control and 36.0±0.5 days in the experimental group.

The organ weights at autopsy are shown in Tables 1 and 2. Both the females (groups 1 and 2) and the males (groups 3 and 4) showed no significant difference as compared with respective control. The ovaries and the testes were normal in histology and exhibited no difference in every respect between control and experimental animals.

Table 2
Body weights and organ weights per 100 g body weight in male rats of groups 3 and 4.

Groups	No. of rats	Body weight	Testes	Seminal vesicles	Coagulating glands	Ventral prostates	Dorsolateral prostates	Adrenals	Hypophysis
		mean±S.E. (g)	mean±S. E. (mg)						
(Group 3)									
Sham-operation	8	425.0±11.6	644.9±31.0	109.5±5.1	32.2±2.3	117.6±5.5	94.6±3.2	13.0±0.5	2.3±0.1
Pinealectomy	7	405.7±21.3	611.8±28.1	121.0±5.3	36.2±3.8	128.0±6.8	98.3±5.1	13.6±0.5	2.5±0.2
(Group 4)									
Sham-operation	9	452.2± 9.9	583.6±14.1	102.4±3.3	23.8±1.0	106.8±2.9	88.6±3.5	10.7±0.4	1.9±0.1
Pinealectomy	10	428.0± 8.5	614.7± 9.8	108.9±4.1	25.6±1.7	115.6±6.1	86.4±3.6	11.0±0.5	2.0±0.1

DISCUSSION

Kincl and Benagiano (1967), who pinealectomized one- and five-day-old rats and studied their reproductive function, stated that the pinealectomy had no influence on the incidence of estrus and the reproductive organ weights, though the opening of the vagina was accelerated 8 to 9 days.

Our results, in the rats housed under cyclic illumination, showed no significant difference between the sham-operated and the pinealectomized rats in every respect; namely, the age of vaginal opening, the ratio of estrous- to total-days and the weights of the reproductive organs. In addition, both groups formed the normal 4-day estrous cycle soon after the vaginal opening and continued to maintain the normal cycle, though the establishment of the 4-day cycle was significantly accelerated in the pinealectomized rats.

Moreover, the rats brought up from birth under continuous illumination also maintained the estrous cycle regardless of the presence of the pineal body throughout the experimental period. A ratio of the normal estrous cycles to all cycles showed no significant difference between the control group and the experimental group. No difference was also found in every index on sexual activities. Like females, the sexual index in males exhibited nothing different between sham-operated and pinealectomized rats.

Accordingly, it is conceivable that the development of the pineal gland has no influence not only on the induction of persistent estrus of rats neonatally exposed to continuous illumination, but also on the formation and the maintenance of the estrous cycle. In our previous paper (Takeo *et al.*, 1975 a), we stated in the adult rats that the pineal circadian rhythm could not directly regulate the estrous cycle, and that the persistent estrus might be induced by the direct action of continuous light stimulation from retina to hypothalamus via optic nerve regardless of the presence of the pineal gland. The present experiment in the neonatal pinealectomy also revealed the similar results. These results mean that the influence of light via the pineal does not play an important role in the induction or the destruction of the sexual cycle in the rats.

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