Timing of sexual receptivity and the release of gonadotrophins during puberty in female rats

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Summary. In about 60% of rats that were paired with fertile males before vaginal opening, vaginal opening occurred between 15:00 and 21:00 h under controlled lighting conditions (lights on 06:00–18:00 h). No rats mated before 12:00 h or after 03:00 h. Most of the rats (52/82) mated within 3 h after vaginal opening. About 90% of the rats that were paired with fertile males before vaginal opening mated and most conceived. Serum LH and FSH levels rose from 14:00 h to a peak at 18:00 h, whereas hypothalamic LHRH content suddenly decreased by 18:00 h.

This study shows that the timing of sexual receptivity, ovulation and the release of gonadotrophins during puberty are similar to those at pro-oestrus in adult rats and suggest that the diurnal rhythm of hormonal changes plays an important role in timing of the first oestrus.

Introduction

In female rats, the first sexual receptivity and ovulation occurs close to the time of vaginal opening (Blandau & Money, 1943). Many investigators have studied the onset of puberty in rats and have shown a relationship between vaginal opening and gonadotrophin release (Ramirez, 1973). According to Nelson, Cullin & Hoffmann (1978) and Ramaley (1979), vaginal opening is useful for the determination of puberty in rats but does not always correspond to the time of the first oestrus.

This study was performed to clarify the timing of vaginal opening, sexual receptivity, ovulation and gonadotrophin release at the first oestrus in female rats.

Materials and Methods

Animals. Wistar–Imamichi strain rats inbred at our centre were used. They were housed in a room with controlled temperature ($23 \pm 2^\circ\text{C}$), humidity (40–60%) and lighting (lights on 06:00–18:00 h). They received dry pellets (CE-2, CLEA, Japan) and tap water *ad libitum*. All females were weaned at Day 21 or 22 of age (Day 0 = day of birth).

Detection of sexual receptivity. Sexual receptivity in 82 females was checked every 3 h from 09:00 h on Days 34 to 38. Two immature females were caged with two fertile males. The first time a copulation plug or spermatozoa were found was designated the time of sexual receptivity (0 h). Of these females 35 were killed at 2-h intervals for hormone determination from 18:00 h. In another group, 117 females were checked at 09:00, 12:00, 15:00 and 18:00 h every day from Days 31 to 42 for spontaneous vaginal opening.

Reproductive performance of pubertal females. In another examination, 113 immature females were caged with fertile males (2 females and 2 males) before vaginal opening on Day 33 of age; 92 which showed oestrus at vaginal opening were examined for fertility. Another 47 females, which

were checked for vaginal opening every 3 h from 09:00 h on Days 33 to 38, were caged with fertile males just after vaginal opening. Both groups were examined at 09:00 h every morning. The day of detection of a plug or spermatozoa was designated Day 1 of pregnancy. Most of the mated rats in both groups (47 and 18) were killed on Days 14–22 of pregnancy, and the number of implantation sites was counted.

Collection of sera and hypothalamic samples. After vaginal opening accompanied by sexual receptivity, 35 rats were killed at 18:00, 20:00, 22:00, 24:00, 02:00, 04:00 and 06:00 h by decapitation. There were few rats in which the vagina opened before 18:00 h. Therefore, immature females with closed vaginas were selected at random from 10:00–16:00 h based on the uterine weight (over 150 mg) and vaginal pro-oestrus (the vagina was artificially opened). Sera were collected and stored at -20°C until assayed for hormones. Immediately after decapitation, the preoptic and hypothalamic areas (about 20 mg) were removed (Glowinski & Iversen, 1966) and boiled in 2 ml 0.1 N-acetic acid for 10 min. They were then homogenized in 5 ml acetic acid solution, and frozen and thawed twice. After centrifugation at 3000 g for 30 min at 4°C , the supernatants were stored at -20°C until assayed for LHRH.

Radioimmunoassay of hormones. Serum LH and FSH were determined according to the directions provided with the NIAMDD rat radioimmunoassay kit. The antisera used were anti-rLH-S6 and anti-rFSH-S11. Preparations used for iodination were NIADDK-rLH-I5 and NIAMDD-rFSH-I5. Results were expressed as ng equiv. RP-1/ml serum. LHRH content in the hypothalamus was determined by a double-antibody radioimmunoassay system, which was developed by Hasegawa, Miyamoto, Yazaki & Igarashi (1981), and expressed in terms (ng/hypothalamus) of synthetic LHRH (Abbott, 26-306AL). Samples were assayed in duplicate. The intra-assay coefficients were 4.0% for LH, 5.1% for FSH and 2.8% for LHRH, and the lower limits of assay for LH, FSH and LHRH were 1.3 ng, 10 ng and 40 pg per tube, respectively.

Statistical analysis. Statistical differences were assessed using Student's *t* test.

Results

Timing of vaginal opening

Spontaneous vaginal opening occurred from Day 31 to 42 of age (means $\pm$ s.d., 36.6 ± 2.7 days, $N = 117$). The numbers of females exhibiting vaginal opening with respect to the time of day, irrespective of the day of vaginal opening, were: 80/117 at 09:00 h, 8/117 at 12:00 h, 9/117 at 15:00 h and 20/117 at 18:00 h.

In rats that were paired with males before vaginal opening, the day of vaginal opening (mean $\pm$ s.d.) was 36.1 ± 1.1 ($N = 82$), with 2 rats showing opening at 03:00 h, 1 at 06:00 h, 3 at 09:00 h, 12 at 12:00 h, 7 at 15:00 h, 29 at 18:00 h, 23 at 21:00 h and 5 at 24:00 h (Text-fig. 1).

Mating and ovulation

As shown in Text-fig. 1, in 70 of the 82 females that were paired with males before vaginal opening, mating occurred within 60 h after vaginal opening, i.e. 33 mated at opening, 19 mated by 3 h and the remaining 18 mated by 60 h. The others (12) did not mate until 72 h. According to the hour of the day, no rats mated before 12:00 or after 03:00 h. The number of mated rats per total number mated was 6/70 at 15:00 h, 25/70 at 18:00 h, 27/70 at 21:00 h, 11/70 at 24:00 h and 1/70 at 03:00 h.

In 4 of the 5 rats examined at 24:00 h, ovulation had occurred during the first oestrus and after that all females ovulated (5/5, 5/5 and 5/5 at 02:00, 04:00 and 06:00 h, respectively). The mean numbers of eggs ovulated per rat were 12.0 ± 0.8 , 13.2 ± 1.0 , 11.6 ± 0.8 and 12.0 ± 1.4 , respectively.

Text-fig. 1. Time of mating after vaginal opening in rats. Boxes indicate the period of vaginal opening for Groups A-H in 3-h intervals. Numbers indicate the numbers of rats mated during every 3-h interval. Group I indicates the period of sexual receptivity for adult rats, i.e. when 22 adult females were caged with males at 09:00 h (↓*) on the day of pro-oestrus, all rats mated by 18:00 h in the same day. Also, 2/7 females that were caged with males at 03:00 h (↓**) on the day of oestrus mated by 06:00 h. Not mated represents the number of rats which did not mate within 72 h after vaginal opening in each group.

In a different experiment, of 92 rats that were paired with males before vaginal opening showed vaginal oestrus, 80 mated within 2 days. Only 47 of these were killed for examination; 39 had conceived and the mean number of implantation sites was 12.1 ± 1.7 . However, only 18 of the 40 rats that were paired with males after vaginal opening mated within 3 days. Of this 18, 16 had conceived and there were 11.0 ± 1.2 implantation sites.

Serum LH, FSH and hypothalamic LHRH content

LH and FSH values began to rise at 14:00 h and reached the maximum at 18:00 h. LH returned to basal levels at 24:00 h, but FSH was still high by 06:00 h the following day (Table 1).

LHRH content was 2.4 ng/hypothalamus at 16:00 h, but this then fell at 18:00 h (1.5 ng) and remained at this low level (Table 1).

Table 1. Changes in serum LH, FSH and hypothalamic LHRH content shortly before and after first oestrus

Time of day (h)	No. of rats	LHRH (ng/hypothalamus)	LH (ng/ml)	FSH (ng/ml)
10:00	8	2.2 ± 0.3	$33.8 \pm 11.6\%$	144.0 ± 16.7
12:00	7	2.4 ± 0.2	56.5 ± 5.9	139.3 ± 15.0
14:00	9	2.3 ± 0.3	554.2 ± 266.4	343.2 ± 144.7
16:00	10	2.4 ± 0.2	$675.7 \pm 217.4^{**}$	$610.4 \pm 190.3^*$
18:00	5	$1.5 \pm 0.2\ddagger$	$702.1 \pm 189.1^{**}$	$849.7 \pm 91.7^{**}$
20:00	5	$1.6 \pm 0.2\ddagger$	$221.9 \pm 39.9^{**}$	$766.8 \pm 61.6^{**}$
22:00	5	$1.5 \pm 0.1\ddagger$	$176.1 \pm 39.1^{**}$	$774.0 \pm 34.7^{**}$
24:00	5	$1.7 \pm 0.1\ddagger$	34.1 ± 12.8	$698.5 \pm 64.4^{**}$
02:00	5	$1.1 \pm 0.1\ddagger$	26.5 ± 6.5	$505.5 \pm 99.9^{**}$
04:00	5	$1.4 \pm 0.2\ddagger$	18.8 ± 2.9	$554.5 \pm 20.8^{**}$
06:00	5	$1.4 \pm 0.2\ddagger$	12.5 ± 1.0	$388.8 \pm 45.0^{**}$

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

§ In 6 rats values were detectable but in 2 they were not ($n = 6$).

Significantly different from the value for 10:00 h, * $P < 0.05$, ** $P < 0.01$.

Significantly different from the value for 14:00 h, † $P < 0.05$, ‡ $P < 0.01$.

Discussion

The present study has demonstrated that the timing of vaginal opening, sexual receptivity and ovulation were closely related to the diurnal changes in hormones. These findings are similar to those reported for adult pro-oestrous rats (Nequin, Alvarez & Schwartz, 1975).

Mating occurred from 12:00 to 03:00 h. This period was consistent with that found by Blandau & Money (1943), but there are some discrepancies which seem to be related to the difference in the method used to assess sexual receptivity. The difference in the distribution of the number of vaginal openings in rats based on the time of day between the spontaneous and paired groups confirmed these discrepancies. These findings also imply that sexual receptivity begins before the complete perforation of the vagina.

The changes in gonadotrophins during puberty have been studied by several investigators (Meijs-Roelofs, Uilenbroek, DeGreef, DeJong & Kramer, 1975; Ojeda, Wheaton, Jameson & McCann, 1976; Dussault, Walker, Dubois & Labrie, 1977; Sarkar & Fink, 1979), but the relationship between the timing of vaginal opening, mating and the changes in gonadotrophins is not clear. Chronological changes in these elements are basically similar to those of adults in pro-oestrus except for LHRH (Daane & Parlow, 1971; Butcher, Collins & Fugo, 1974; Araki, Ferin, Zimmerman & Vande-Wiele, 1975; Nequin *et al.*, 1975; Chiappa & Fink, 1977). LHRH content in the hypothalamus after LH release during the first oestrus was still low compared with that in adult rats. This discrepancy may be related to differences in rates of synthesis of LHRH (Ramirez & Sawyer, 1966; Ojeda *et al.*, 1976; Sarkar & Fink, 1979; White & Ojeda, 1981; Advis, Krause & McKelvy, 1982).

Compared to cyclic rats, ovulation at first oestrus seems to occur slightly earlier, and the "critical period" for the release of gonadotrophins seems to be wider.

LHRH may play an important role in the initiation of vaginal opening (Sakuma & Pfaff, 1980), because the release of LHRH appears to be associated with opening of the vagina. However, the timing of vaginal opening may be influenced by the diurnal rhythm of endocrine changes during the prepubertal period (Döcke & Dörner, 1974; Firlit & Schwartz, 1977; Ronnekleiv, Ojeda & McCann, 1978; Kraulis, Naish, Gravenor & Ruf, 1981).

Most of the rats mated during the first oestrus conceived. This finding coincides with other reports (Blandau & Money, 1943; Miller & Armstrong, 1981), but not with that of Advis & Ramirez (1977). Vaginal opening and sexual receptivity occur within a short period, and mating rates are therefore related to the time at which the female rats are paired with males. These results show that pubertal females have the same reproductive ability as adults, but the continuation of pregnancy and fetal development in pubertal rats remains to be studied.

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