Nest Building Behavior of Mouse and Effect of Ovarian Hormones on the Behavior

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Summary

The correlation between nest constructing behavior and stages of oestrus cycle and pregnancy and effect of ovarian hormone treatment on the behavior were studied in mice. Nest constructing behavior was sevearly affected by the circulating level of sexual hormones. Metoestrus animals displayed a significant increase of nest preparing activity compared with the activity of oestrus and proestrus animals. In pregnant mice, the nest weight started to increase markedly from day 4, reached a peak level on day 7 and decreased from day 14 of pregnancy. Nest preparation was evoked by exogenous administration of ovarian hormones in either intact or ovariectomized mice. The largest nest was observed in animals treated with 1 mg progesterone and 20 ng estradiol daily and the animal treated with progesterone alone followed. No induction of nest building was observed in animals treated with progesterone and 100 ng estradiol and estradiol along. Nest preparation in mouse may be facilitated by a synergim between small amount of estrogen and large amount of progesterone.

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Key words : mouse, nest building, ovarian hormone

Introduction

Adult female mice are able to construct two different types of nest depending upon their reproductive state[1,2]. One type of nest called as saucer nests or "sleeping nests" is relatively small, and constructed by nonpregnant animals. The other type reffered as brood nests or "maternal nests" is prepared by pregnant mice. This type of nest is approximately two to three times biggers than the first type, and completely enclosed, like a ball having two or more entrance tunnels usually.

Nest building as a maternal behavior was first observed by Kollor[1,2], in ovariectomized females administered with progesterone. Previous reports indicated that maternal

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behavior about nest preparation would depend on an adequate titre of progesterone and estrogen, although sometimes nest preparation was performed by ovariectomized mice treated with progesterone only[3-6]. However, few experiments have been made about the nest constructing behavior of mice in reference to naturally occuring reproductive events. Lisk et al.[3] could find no special correspondence between nest-preparation performance of non-pregnant mice and their oestrous cycle. The workers used hay as nesting material, Zarrow[7] used cotton instead of hay. Usually the cotton was placed in a receptacle at the top of the cage and pulled into the cage to be incorporated into a nest. Thus, the amount of cotton pulled into the cage is a reliable indicator of nest size and hence the nest preparation activity of animals together with the nest style they form.

The present study consisted of three experiments. Experiment 1 was performed to establish the change in nest preparation behavior during oestrous cycle. Experiment 2 clearly demonstrated the occurance of pre-partum maternal behavior. And experiment 3 was performed to determine possible steroid effects by altering the hormonal milieu on the final day of gestation.

Materials and Methods

The animals wer of CFW strain maintained in our laboratory as a closed colony. Virgin females at 7 weeks of age (body weight 18-22 g) were housed singly in 24.5×7.5×12.5 cm plastic cages with a wire top. The cage floors were covered with sawdust. The animals were fed ad libitum and maintained under controlled temperature (20 °C) and lighting (12 h light and 12 h darkness, lights-on at 6.00 AM) conditions.

A pre-weighed ball of cotton was placed into the food basket at the top of the cage. Mice were allowed to builed nests freely, the cotton pulled by each mouse into the cage for the nest preparation during a 24 h period was weighed and the style or type of the nest made was recorded. The time of weighing of cotton pulled and observation of nest type was approximately the same every day. After the nest was removed, the cage was cleaned and a new cotton ball was placed daily at 9.00 AM. The nest style was classified into three different categories (Fig 3) according to Lisk et al.[2,4], i. e., the cotton spread all over the floor (type A), the bird type nest with wall (type B), and the maternal nest with roof (type C). The B and C type nests differ each other in the height of the wall of cotton.

Experiment 1. Fifty-seven mice were used as subjects. All animals were checked for their estrous cycle by vaginal smearing, but the cycles are usually irregular. Therefore, the nest preparing behavior was observed for consecutive 10 days irrespective of their cycle and the data obtained were arranged according to the stage of estrous cycle judged from vaginal smear on the day.

Experiment 2. Ten mice were used and vaginal smear was examined daily for 2

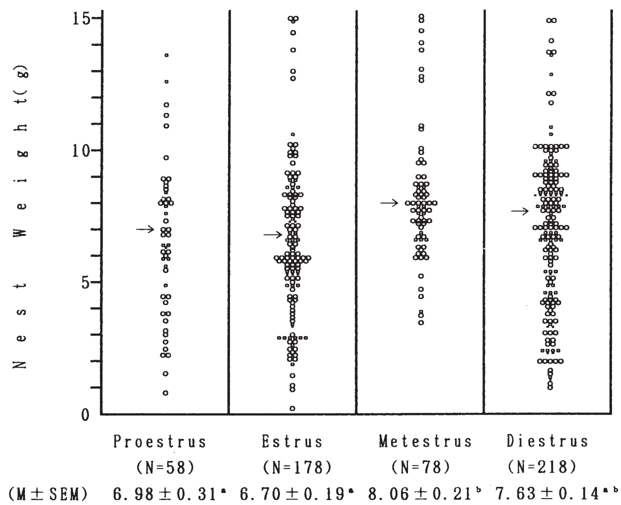

Fig. 1 The weight of nest formed by virgin mice at each stage of vaginal estrous cycle.
→ : Data are the mean±SEM.
ab Dissimilar letters indicate a significant difference ($P<0.05$).

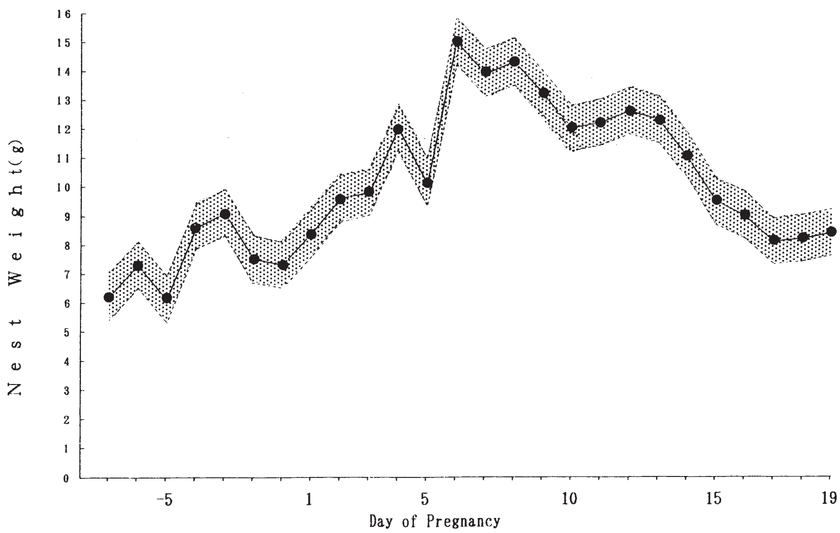

Fig. 2 The average weight of cotton pulled into the cage daily (nest weight) during pregnancy ($n=10$)
● : Data are the mean±SEM.

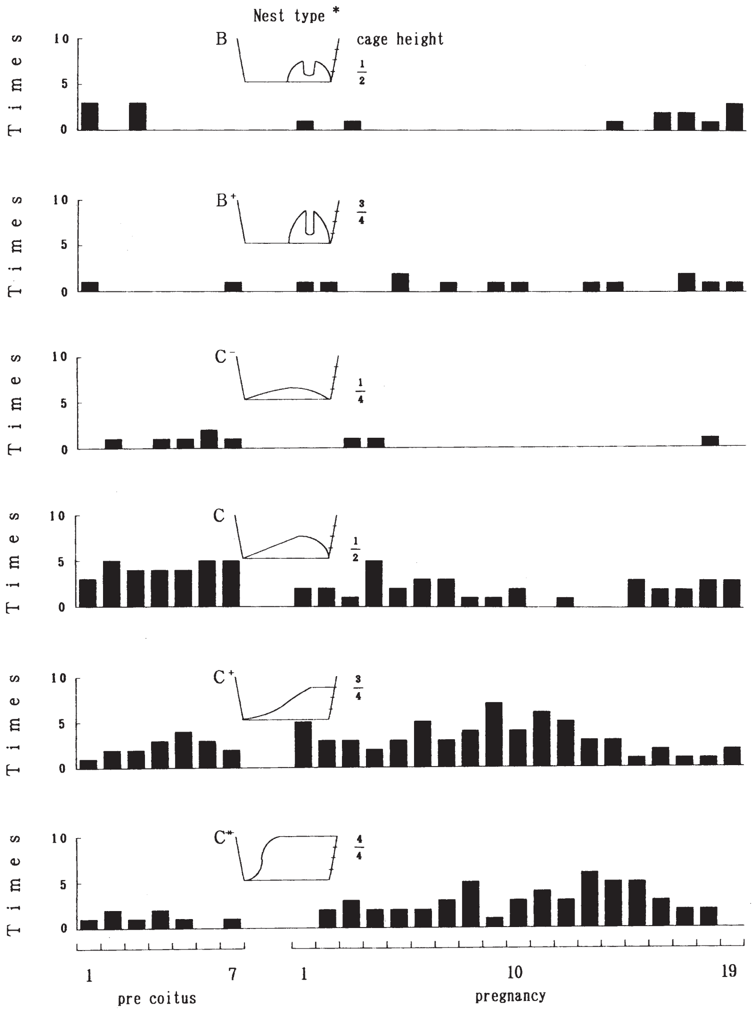

Fig. 3　Incidence of different types of nest during estrus cycle and pregnancy
＊ The nest types are judged according to the classification of Lisk et. al. (1969).

weeks before the start of experiment. On the day of vaginal proestrus female mice were cohabited with males. The day on which vaginal plug was observed designated day 1 of pregnancy. The nest preparation behavior was observed for 7 days before mating and from day 2 to day 21 of pregnancy.

Experiment 3. Overiectomy of mice was performed at 7 weeks of age under light ether anesthesia. One week after the operation, overiectomized and sham ovariectomized animals and intact animals of the same age, 57 in total, were assigned to the following 7 treatment groups (7-9 mice/group) ： group A, sham operated controls ； group B, ovariectomized (OVX) controls ； group C, OVX plus 1 mg progesterone (P_4) ； group

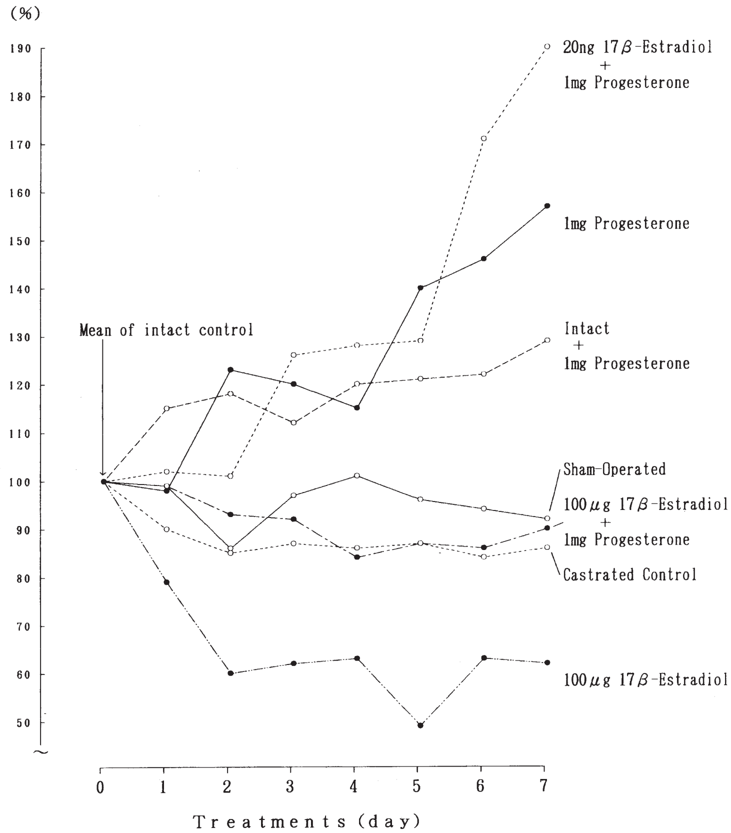

Fig. 4 The average amount of cotton daily pulled by each group of ovariectomized intact control or ovarian hormone treatd group of mice.

D, OVX plus 100μg 17β-estradiol (E_2) ; group E, OVX plus 100μg E_2 and 1 mg P_4 ; group F, OVX plus 20 ng E_2 and 1 mg P_4 and group G, intact animals treated with 1 mg P_4. The hormones were dissolved in 0.05 ml sesame oil and administered subcutaneously once a day for consecutive 7 days. To the group Aand B same volume of the oil alone were given. The nest preparation behavior was observed for one week before the operation, one week from the day of ovariectomy and one week after the hormone treatment finished.

For statistical analysis Student's t test was used.

Results

Experiment 1 : The relationship between the nest weight formed by virgin mice and the stages of oestrous cycle was shown in Fig 1. The oestrous cycle of mice used was not regular and their the number of data varied greatly among the stages. The nest weight was less on days of vaginal proestrus and oestrus and greater on metoestrus and diestrus days. The weight was maximum at metestrus and differs significantly from that of proestrus and oestrus period ($P<0.05$).

Experiment 2 : The average weight of cotton pulled into the cage daily during pregnancy is shown in Fig 2. A marked rise in nest preparation activity began on day 4 of pregnancy and significantly differ from control ($P<0.05$). The nest weight continued to increase thereafter, reaching on day 7 and then decreased gradually to days 17-19 of pregnancy ($P<0.05$). On 19 days of pregnancy, on which the weight returned to the level comparable to that during oestrous cycle.

Fig 3 illustrates the incidence of each nest type in virgin 70 nests in total and pregnant 126 nests in total mice. I can not observed A and B-type nest. The nest formed by virgins are generally smaller and more compact than those formed by pregnant animals as bird type nest. Pregnancy showed a substantial increase of nest weight as well as percent of maternal types. Maternal nest weight were reached a peak on day 10 of pregnancy and maternal nest number were reached in peak on day 14 of pregnancy.

Experiment 3 : The mean amounts of cotton daily pulled by each group are presented in Fig 4. Female mice administered with 20 ng 17 β-estradiol plus 1 mg progesterone (group F) showed a highly significant increase of nest weight compared with control ($P<0.01$). During this treatment the most of nest were observed maternal style and pregnancy (42.0 %) was higher than intact (35.0 %) in pregnancy group. The mean nest-weight of females tended to increase some what after administration with 1 mg progesterone (group C) and intact plus 1 mg progesterone (group G) ($P<0.05$). Among all group of females administered with 100μg 17β-estradiol (group D & E) the mean nest-weight decreased significantly compared with control ($P<0.01$).

Observation of nest types showed that the normal control animals built mainly pallet-nest, half-nest and bird-nest. After ovariectomization, the type of half-nest and bird-nest decreased and the type of pallet-nest and pocked palleted nest increased. The nest size of ovariectomized mice tend to built small. The group F, G and C mice built large maternal nests, whereas the group D and E mice typically constructed small, saucer-shaped sleeping nest.

Discussion

Several behavior during pregnancy may be considered maternal or prematernal. Present study established the corelation between them by using cotton as nesting material. The significant increase of nest-preparating ability of mouse observed in this experiment during pregnancy were in aggreement with other researchers[3,8]. During pregnancy mice and rats construct larger and more elaborate nests than do nonpregnant animals. In the mouse this increase occurs four or five days after mating, a time which coincides with the appearance of ovarian corpora lutea and beginning of the progestational state of pregnancy[1,2].

The formation of a corpus luteum and secretion of progesterone depend upon stimulation of the ovary by pituitary gonadotropic hormones, particularly prolactin and lutenizing hormone (LH). A phasic realese of these hormones follows mating[8,9]. An elevation of prolactin appears necessary for initiation of the luteal phase of ovarian function in the mouse and rat, and its release from the pituitary is partly dependent upon vaginal stimuli during mating[10]. LH further stimulates the synthesis and secretion of progesterone, and low but tonic levels of LH during the first half of pregnancy are apparently necessary to maintain corpus luteum activity[11]. Deprivation of LH, by administration of an LH antiserum for even a few hours interferes with corpora luteum activity and may result in death and resportion of the fetuses. This dependence upon LH apparently accounts for the fact that hypophysectomy before day 12 of gestation invariably results intermination of pregnancy. After day 11-12, hypophysectomy no longer terminates pregnancy because by this time the placenta produce sufficient luteotropic substances to maintain ovarian function[13]. The luteotropic surges responsible for initiation corpus luteum activity are reflected by the initial increase in LH and prolactin. The drop in LH on days 12 and 13 is correlated with an increase in gonadotropic stimulation by the placenta and may reflect a negative feedback by placental secretions on LH release. The precipitous decline in progesterone on day16 to 18, signals the beginning of the preparturient phase of pregnancy[9].

From this event, the relationship between nest-preparating behavior and pregnancy is congruent with what is known about the endocrine events surrounding pregnancy[8]. Then, progesterone is apparently responsible for eliciting the preparation of maternal nests. Exogenous progesterone given to virgin female mice causes an increase in nest building after two to three days[1-3]. Progesterone treatment is also effective in ovariectomized, but the greatest faciliation of nest building in castrates is obtained when small priming doses of estrogen are given before progesterone[4], this is in accord with the known synergistic action of these two steroids.

In this experiment, the maximum nest weight in that administered with 20 ng 17β-estradiol plus 1 mg progesterone than overiectomized group, administered with 1 mg progesterone alone. It is probable that progesterone must synergize with small amounts of oestrogen in order to produce maximum nest-preparing ability was also found by Lisk et al.[5]. When the amount of 17 β-estradiol administered 100μg, oestrogen acting alone significantly decreased nest weight. Large amounts of oestrogen can block nest-preparating ability and that were similar to those described by Lisk[4]. Slotnick et al.[13] used mouse and they confirmed that the amount of material used and each built a large nest with high walls and partial celling by treating with progesterone.

Reference

1) Kollor, G. (1952) Der Nestbau der weiber Maus und seine hormonai Auslosung. Verh. dt. Zool. Ges., 160-168.

2) Kollor, G. (1955) Hormonale und psychishe Steuerung beim Nestbau weiber Mause. Zool. Anz (Suppl.), 19 : 125-132.

3) Lisk, R. D., Pretlow, R.A. & Freidman, S.M. (1969) Hormonal stimulation necessary for elicitation of maternal nest building in the mouse (*Mus musculus*). Anim. Behav., 17 : 730-737.

4) Lisk, R. D. (1971) Oestrogen and progesterone synergism and elicitation of maternal nest-building in the mouse. Anim. Behav., 19 : 606-610.

5) Lisk, R. D., Russell, J.A., Kohler, S.G. & Hanks, J.B. (1973) Regulation of hormonally mediated maternal nest structure in the mouse (*Mus musculus*) as a function of neonetal hormone manipulation. Anim. Behav., 21 : 296 -301.

6) Gardelman, R. (1975) Maternal nest building performance and fetal number in Rockland Swiss albino mice. J. Reprod. Fert., 4 ： 551-554.

7) Zarrow, M.X., Faroog, A., Denenberg, V.H., Swain, P.B. & Ross, S. (1963) Maternal behaviot in the rabbit : endocrine control of maternal nest building. J. Reprod. Fert., 6 : 375 -383.

8) Linkie, D.M. & Niswender, G.D. (1972) Serum levels of prolactin, luteinizing hormone, and follicle stimulating hormone during pregnancy in the rat. Endocrionology, 90 : 632-637.

9) Morishige, W.K, Pepe, G.J. & Rothild, I. (1973) Serum luteinizing hormone, prolactin and progesterone levels during pregnancy in the rat. Endocrinology, 92 : 1527-1530.

10) Spies, H.G. & Niswender, G.D. (1971) Levels of prolactin, LH and FSH in the serum of intact and pelvic-neurectomized rats. Endocrinology, 88 : 937-943.

11) Raj, H.G.M. & Moudgal, N.R. (1970) Hormonal control of gestation in the intact rat. Endocrinology, 86 : 874-889.

12) Murr, S.M., Stabenfeltdt, G.H., Bradford, G.E. (1974) Plasma progesterone during pregnancy in the mouse. Endocrinology, 4 ： 1209-1211.

13) Slotnick, B.M. (1975) Neural and Hormonal Basis of Maternal Behavior in the Rat. In " Hormonal Correlates of Behavior." ed. Eleftherious, B.E., Sprott, R.L. p. 586-656.

マウスの巣造り行動と同行動に対する卵巣ホルモンの影響

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要 約

マウスの性周期における巣造り行動と卵巣ホルモンの影響について観察を行った。巣造り行動は性ホルモンによって支配された。性周期中の巣造りは，発情後期が最大で発情期および発情前期は最小であった。巣造りの大きさと性ホルモンの関係は，黄体ホルモン下で大きく，発情ホルモン下で小さかった。妊娠中の巣造りは妊娠４日目から増大し，妊娠７日目で最高値を示し，妊娠14日目から減少した（膣栓を発見した日を妊娠１日)。これらの巣造り行動は，他の報告者の血清中の黄体ホルモンとの動きと一致がみられた。また，マウスの母性行動にみられる巣は，少しの発情ホルモンと多量のプロジェステロンの組合せで起こることが判明した。

キーワード：マウス，巣造り，卵巣ホルモン