

The Effect of Pregnancy and Stress on the Onset of Placentophagia in Long-Evans Rats¹

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KRISTAL, M. B., L. C. PETERS, J. R. FRANZ, J. F. WHITNEY, J. K. NISHITA AND M. A. STEUER. *The effect of pregnancy and stress on the onset of placentophagia in Long-Evans rats.* PHYSIOL. BEHAV. 27(4) 591-595, 1981.—Most virgin rats do not eat placenta when it is presented to them; virtually all parturient rats do. This study was designed to examine the role of the duration and termination of pregnancy on the induction of placentophagia. Time-bred rats determined by a pretest not to be attracted to placenta (nonplacentophages), were tested for placentophagia on one of a number of days of pregnancy. A comparable group was tested for placentophagia after surgical termination of pregnancy on Day 21. Groups of virgins were run controlling for the time interval between the pretest and the placentophagia test, and for a time interval that included a "stressful" event. The results were that (a) the incidence of placentophagia rose gradually from Day 7 to Day 15 of pregnancy, then remained stable, at about 0.4, until delivery; (b) pregnancy termination did not produce an effect on placentophagia greater than that of untermiated pregnancy; (c) a pretest-test interval containing a "stressful" event produced significantly more placentophagia than one that did not contain such an event; and (d) the maximum level of placentophagia observed during pregnancy was the same as that produced in virgins by a "stressful" event.

Placentophagia Pregnancy Long-Evans rats Maternal behavior Cesarean section Stress

ALTHOUGH virtually all parturient rats ingest the placenta during delivery [2, 3, 9, 14], few virgins eat donor placenta when it is made available to them [3,4]. Furthermore, the proportion of nonpregnant multiparae willing to eat placenta is larger than that of virgins, presumably because ingesting placenta (placentophagia) during delivery permanently converts some noneaters to eaters [4]. The dramatic change that occurs, from virgin placenta-avoider to avid placentophage during delivery, is very much like the change in general maternal responsiveness that occurs when virtually all nonmaternal virgins become avidly maternal puerpera. In placentophagia, as in maternal behaviors directed toward the neonate (retrieving, licking, crouching, etc.), the behavior is performed enthusiastically, immediately upon presentation of the stimulus, and without benefit of prior experience with that stimulus. The present study had two goals. The first was to investigate the extent to which the change from nonplacentophage to placentophage is associated with progress through pregnancy. This is essentially the same logic used in early investigations of the hormonal basis for the onset of pup-directed maternal behavior in rats [10]. The second goal was to test the possibility that pregnancy and

pregnancy termination have an effect on placentophagia only because they act as general "stressful" events [4,13], and not because the onset of placentophagia is triggered specifically by changes in reproductive physiology.

METHOD

Animals

Four hundred forty-six female Long-Evans rats, 2-3 months old at their entry into the experiment, were tested. The subjects were laboratory-born and -reared daughters of male and female Long-Evans rats purchased from the Charles River Breeding Laboratories; Laboratory-born virgins show a lower incidence of spontaneous placentophagia than do purchased virgins [4]. The rats were housed individually in 24×19×18-cm hanging wire-mesh cages fitted with water bottles and with food hoppers containing Charles River Rat/Mouse/Hamster Formula 3000. When necessary, rats were housed individually in 45×19×25-cm plastic cages containing 3 cm of coarse sawdust. Except when otherwise noted, food and water in both types of cage were available ad

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lib. The colony was maintained on a 14:10 day:night cycle, with the day phase beginning at 0600 hr (EST).

Procedure

Placentophagia pretest. All 446 rats were tested for their response to placenta before entry into the experiment, in a procedure that is now standard in our laboratory [3,4]. Beginning between 60 and 75 days of age, each female was checked for normal estrous cyclicity by daily inspection of vaginal smears. When normal cyclicity was confirmed, each rat received as many as three consecutive daily presentations of donor placenta. Donor placenta was obtained surgically from CO₂-killed, 21-day pregnant rats, and then frozen at -20°C along with a few drops of physiological saline. When needed for testing, vials of frozen placenta were rapidly warmed to 37°C and placentas presented (one or two at a time) in an untipable glass dish to rats that had been without water for 15 min and without food for 2 hr. Placentophagia pretests were conducted during the lights-on period (1200 hr to 1500 hr). If the rat ingested placenta during a 15 min presentation, she was regarded as a placentophage, and the tests discontinued. If three consecutive tests went by without placentophagia, the rat was scored as a nonplacentophage. The behavior of nonpregnant rats is clearly dichotomous. Most refuse to eat placenta, but those that are or become placentophages, remain placentophages [4]. All 446 rats in the present study were determined by this pretest to be nonplacentophages, and in the process had received the maximum number of exposures to placenta, three. When tested subsequently for placentophagia in the various experimental conditions, therefore, the rats were being presented with placenta for a fourth time some weeks after the 3-day pretest. Placentas were presented without pups attached since previous work in which a pup+placenta stimulus was used indicated that the response toward each component was different and was confounded by the presence of the other. Furthermore, a substantial proportion of virgins presented with pups and placenta still refused to eat the placenta [8,12]. Our feeling was that to understand the response of females to birth material, therefore, it was critical to test the response to only one substance at a time.

Placentophagia during pregnancy. Each of 233 nonplacentophages was time-bred to a proven breeder male and given a single 15-min placentophagia test on a pre-determined day of pregnancy. A minimum of 12 rats was tested on each test day. After the test, the female's delivery date was noted and the test then recomputed as having occurred the observed number of days prior to delivery. Time-breeding was carried out 1 to 2 weeks after the pretest; therefore, the pregnancy test was a fourth placenta presentation occurring 2 to 6 weeks after the initial three presentations.

Of the 44 rats tested for placentophagia on the last day of pregnancy, 29 were observed for the exact hour of delivery, thus allowing us to compute the number of hours prior to delivery that the tests occurred. The last 24 hr of pregnancy was then blocked into four 6-hr units. Normative data for placentophagia during delivery, in the absence of previous placentophagia tests, were accumulated over the last several years from observations of delivery in untreated rats in our laboratory. Virtually 100% of such animals displayed placentophagia during parturition. This is consonant with comparable observations in other strains of rat [2, 9, 14].

Twelve nonplacentophages were impregnated and

allowed to deliver without a placentophagia test during pregnancy. Two hours after delivery was completed, however, they were presented with a dish containing two donor placentas to compare their response to donor placenta with that of the group tested immediately before delivery, and to compare the response of these postpartum females toward donor placenta with their response toward their own freshly delivered placenta.

Pregnancy termination and placentophagia. The rapid onset of intense, pup-directed maternal behavior that is observed at parturition occurs not just after a full-term pregnancy, but more specifically, after the termination (by delivery) of a full-term pregnancy. Experiments testing the effect of terminating pregnancies prematurely by surgical intervention demonstrated that beginning about halfway through pregnancy, surgical removal of the fetuses causes very rapid development, in the mother, of maternal behavior toward foster pups [6, 7, 10]. Intact pregnant rats, tested at the same stage of pregnancy, do not show that same high level of maternal responsiveness [6,10]. Therefore, although the potential for pup-directed maternal behavior exists, it is not seen without a specific experimental intervention until the last day or so before delivery. We sought to determine whether pregnancy termination, produced surgically, could also increase the likelihood of placentophagia beyond the level produced by the duration of pregnancy.

Seventeen pregnant nonplacentophages received cesarean sections on Day 21 of pregnancy, under methoxyflurane anesthesia (Metofane, Pitman-Moore). After surgery, the females, but no pups, were returned to their plastic cages. Either 6 hr after surgery (n=10) or 24 hr after surgery (n=7), each female was presented with a donor placenta in a glass dish for 15 min. The proportion of placentophages in each group was noted.

Placentophagia in unstressed virgin controls. All placentophagia tests in pregnant rats actually consisted of a fourth placenta presentation occurring some weeks after the three consecutive exposures constituting the pretest. Therefore, fourth-test data from non-impregnated females had to be obtained for comparison. Ninety-one virgin nonplacentophages were maintained under routine laboratory conditions. Handling was kept to a minimum. About one-third of the group was pair-housed, the remainder housed individually. Seventy of the rats were given their fourth placenta presentation (placentophagia test) at a precise interval after the pretest: 1 wk (n=14), 2 wk (n=15), 3 wk (n=16), 4 wk (n=14), 5 wk (n=11). The remaining 21 rats were tested at less precise intervals after the pretest: 1-2 wk (n=7), 2-3 wk (n=7), 3-4 wk (n=7).

Placentophagia in "stressed" virgin controls. Kristal and Graber [4] noted that the proportion of placentophages in a group of Long-Evans females obtained from a commercial supplier was significantly greater than that of a comparable group born in the research laboratory. They hypothesized that the difference may have been due to the additional, and perhaps stressful, experience the purchased rats had had, of being crated and shipped from the breeding facility to the research laboratory (see also [13]).

If the experience of being shipped was sufficient to elevate the likelihood of placentophagia in a group of virgins, it was distinctly possible that mating, or pregnancy, or both, might also be an intense enough experience to increase the likelihood of placentophagia in a group of nonplacentophages. A resulting increase in placentophagia associated with pregnancy, then, might actually be due to an indirect,

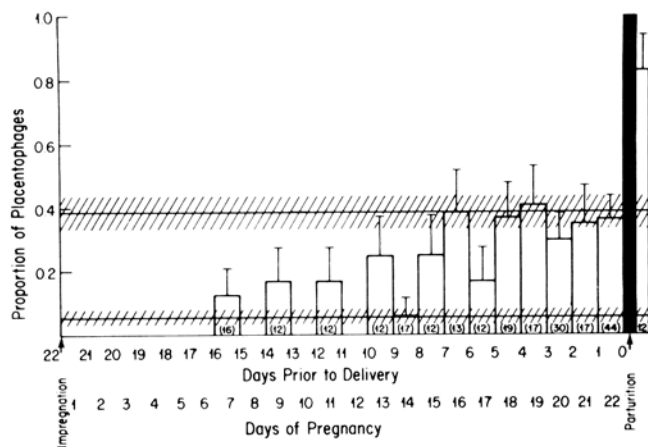


FIG. 1. Proportion (\pm SEP) of placentophages in groups of pregnant nonplacentophagic rats tested for response to donor placenta on one day of pregnancy, compared with the base level proportion for "stressed" virgins (0.387 ± 0.051) and for unstressed virgins (0.044 ± 0.021). The number of rats tested each day is in parentheses.

general effect, rather than a specific effect produced by changes in reproductive physiology. We decided, therefore, to compare the response of the previously described groups with that of virgins tested in our laboratory, over the past several years, after a variety of experiences that can arguably be classified as stressful. Like the previously described groups, all 93 of these rats had been determined, in our standard 3-day pretest, to be nonplacentophages, and had received a fourth exposure to placenta (placentophagia test) 4 wk later. The presumably stressful experience occurred during the first 2 wk of that 4 wk interval. These experiences were as follows:

- Sham ovariectomy (i.e., a laparotomy)+stereotaxically produced septal lesion (both bilateral), $n=10$.
 - Ovariectomy+sham septal lesion, $n=10$.
 - Ovariectomy+septal lesion, $n=10$.
 - Sham ovariectomy+sham septal lesion, $n=10$.
- The operations for groups (a) through (d) were performed under pentobarbital/chloral hydrate anesthesia (Chlorpent, Fort Dodge Laboratories, 3 ml/kg body weight, IP).
- Ovariectomy under ether anesthesia, $n=15$.
 - Injection of common vehicles (0.4 ml normal saline, IP; 0.4 ml distilled water, IP; 0.1 ml olive oil, SC; 0.25 ml ethanol, IP), $n=28$.
 - Crating and shipping, $n=10$.

In this last case, 7-wk-old virgin Long-Evans females, that had been born in our laboratory, were placed in a Charles River Breeding Laboratories shipping carton, put in a car, driven around town for three hours (on a spring day), and delivered to a similar laboratory colony in a different building. Upon arrival in the new colony, the rats were unpacked, housed, maintained, and eventually tested for placentophagia, in a manner identical to that used for rats in our laboratory that are purchased from a commercial breeder.

RESULTS

Placentophagia During Pregnancy

Not all rats have 22.5-day pregnancies, but for purposes

of exposition, the day of pregnancy on which a test occurred is referred to as the day of pregnancy it would have been, had it occurred in the modal 22.5-day gestation. For precision, however, when necessary the number of days from test to delivery was actually observed, and the time was recorded as the number of days prior to delivery. Therefore, the time scale on Fig. 1 that is adjusted to parturition is more accurate than that adjusted to impregnation.

Placentophagia tests were conducted on Days 7, 9, 11, and 13 through 22 of pregnancy. The proportion of the group tested on each day that exhibited placentophagia is depicted in Fig. 1.

A one-way ANOVA for dichotomous (0,1) data [5] comparing the proportion of placentophages on various days of pregnancy, indicated that there were no significant differences among the days, $F(12,220)=1.12$, $p>0.05$. There was, however, a significant rank-order correlation between the day of pregnancy and the proportion of placentophages found; the later the day, the higher the proportion (Spearman $\rho=.699$, $z=2.42$, $p<0.01$).

Ten of the 12 rats presented with donor placenta after delivery ate it. This proportion was significantly greater than the highest proportion seen during pregnancy (0.833 ± 0.108 vs 0.412 ± 0.119 ; $\hat{\sigma}_{\hat{p}_1-\hat{p}_2}=0.186$, $z=2.27$, $p<0.01$). The proportion is obviously not different from that of parturient rats that ate the fresh afterbirths as they emerged (see Fig. 1), despite the fact that the rats presented with donor placentas ate them in addition to all those they had delivered.

Close examination of placentophagia during the last 24 hr prepartum indicated that universal placentophagia does not appear before the onset of delivery. Of 15 rats given a placentophagia test 0–6 hr prior to delivery, only 3 ate placenta (0.200 ± 0.103); two of the 5 rats tested 7–12 hr before delivery ate placenta (0.400 ± 0.219); two of the 5 tested from 13–18 hr ate (0.400 ± 0.219); and 1 of the 4 tested from 19–24 hr ate (0.250 ± 0.216). Clearly the level of placentophagia seen in the last several hours of pregnancy does not represent the level of placentophagia observed during or shortly after delivery.

Pregnancy Termination and Placentophagia

Ten of the rats receiving a cesarean section on Day 21 of pregnancy were presented with donor placenta 6 hr after surgery. Only 2 rats of this group ate the placenta (0.200 ± 0.126). Four of the 7 rats presented with donor placenta 24 hr after surgery ate it (0.571 ± 0.187). These two groups were not significantly different from each other ($\hat{\sigma}_{\hat{p}_1-\hat{p}_2}=0.236$, $z=1.57$, $p>0.05$). The overall proportion of rats with terminated pregnancies that manifested placentophagia, therefore, was 0.353 ± 0.116 . The proportion of placentophages observed on Day 21 of a non-terminated pregnancy was also 0.353 ± 0.116 (see Fig. 1). Pregnancy termination, therefore, did not elevate placentophagia beyond the level produced by pregnancy.

Placentophagia in Virgin Controls

The overall incidence of placentophagia in virgins in the unstressed condition was extremely low: 4 placentophages of 91 rats tested (0.044 ± 0.021). The incidence of placentophagia in unstressed virgin controls is presented in Table 1. There were no statistically significant differences among the precise interval groups, $F(4,65)=1.14$, $p>0.05$, none among the less precise interval groups, $F(2,18)=1.0$, $p>0.05$, and none between the precise interval groups and the less precise

TABLE 1

INCIDENCE OF PLACENTOPHAGIA IN UNSTRESSED NONPLACENTOPHAGIC VIRGINS ON A TEST THAT OCCURRED AT ONE OF SEVERAL INTERVALS AFTER THE PLACENTOPHAGIA PRETEST

Interval	N-Tested	Proportion \pm SEP Placentophages
1 Week	14	0.000 \pm 0.000
2 Weeks	15	0.067 \pm 0.064
3 Weeks	16	0.125 \pm 0.083
4 Weeks	14	0.000 \pm 0.000
5 Weeks	11	0.000 \pm 0.000
1-2 Weeks	7	0.000 \pm 0.000
2-3 Weeks	7	0.143 \pm 0.132
3-4 Weeks	7	0.000 \pm 0.000
Overall	91	0.044 \pm 0.021

TABLE 2

INCIDENCE OF PLACENTOPHAGIA AMONG NONPLACENTOPHAGIC VIRGINS EXPOSED TO ONE OF A NUMBER OF "STRESSFUL" EXPERIENCES AFTER THE PLACENTOPHAGIA PRETEST

Group	N-Tested	Proportion \pm SEP Placentophages
Ovex-Septalex	10	0.600 \pm 0.155
Sham Ovex-Septalex	10	0.500 \pm 0.158
Ovex-Sham Septalex	10	0.500 \pm 0.158
Sham Ovex-Sham Septalex	10	0.600 \pm 0.155
Ovex (ether)	15	0.133 \pm 0.088
Injections	28	0.214 \pm 0.078
Car Ride	10	0.600 \pm 0.155
Overall	93	0.387 \pm 0.051

interval groups (standard error of the difference between two proportions [1]), 3/70 vs 1/21, $\hat{\sigma}_{\hat{p}_1 - \hat{p}_2} = 0.051$, $z = 0.09$, $p > 0.05$. The group proportions varied from a low of 0 (in four of the groups) to a high of 0.143 (2-3 wk group). Clearly, when exceptional, potentially stressful events do not occur, the proportion of placentophages remains low.

The overall incidence of placentophagia in virgins receiving a presumably stressful manipulation was very high (0.387 \pm 0.051), and was significantly higher than that for the unstressed virgins, 36/93 vs 4/91, $\hat{\sigma}_{\hat{p}_1 - \hat{p}_2} = 0.061$, $z = 5.64$, $p < 0.001$. The proportions of placentophages found after various "stressful" treatments are presented in Table 2. The various treatments within the "stressed" group produced proportions of placentophages ranging from a low of 0.133 (ovariectomy with ether), to 0.600 (both car ride and double sham surgery), but there were no significant differences among the various manipulations, $F(8,102) = 1.69$, $p > 0.05$. Although the proportion of placentophages found in the group receiving an ovariectomy under ether anesthesia (0.133) was only marginally greater than 0 and was in the range of the largest proportions found among the unstressed groups, that group was pooled with the other "stressed" groups in calculating the overall proportion of placentophages, on the basis of the absence of statistically significant differences indicated by the ANOVA. It is important to note that the values obtained for the stressed and unstressed groups in this study are consonant with values for purchased and laboratory-born virgins reported by Kristal and Graber, 0.47 and 0.04, respectively [4].

The proportion of placentophages found on the earliest day of testing, Day 7, was not significantly different from the base level for unstressed virgins (0.125 \pm 0.083 vs 0.044 \pm 0.021; $\hat{\sigma}_{\hat{p}_1 - \hat{p}_2} = 0.062$, $z = 1.299$, $p > 0.05$), but was significantly lower than the base level of placentophagia for "stressed" virgins (0.125 \pm 0.083 vs 0.387 \pm 0.051; $\hat{\sigma}_{\hat{p}_1 - \hat{p}_2} = 0.129$, $z = 2.03$, $p < 0.05$, see Fig. 1). The proportion of placentophages on the last day of pregnancy, i.e., the last day before delivery, was significantly greater than the base level for unstressed virgins (0.364 \pm 0.073 vs 0.044 \pm 0.021; $\hat{\sigma}_{\hat{p}_1 - \hat{p}_2} = 0.065$, $z = 4.90$, $p < 0.01$) and was not significantly different from the base level for "stressed" virgins (0.364 \pm 0.073 vs 0.387 \pm 0.051; $\hat{\sigma}_{\hat{p}_1 - \hat{p}_2} = 0.089$, $z = 0.26$, $p > 0.05$).

DISCUSSION

Virtually all rats giving birth for the first time ingest placenta during delivery, although only a relatively small proportion of virgins eat the substance when it is presented to them. The results of the present study showed that (a) only 4.4% of virgin nonplacentophages became placentophages after several weeks of uneventful laboratory life, but 38.7% became placentophages in the same interval if that interval contained a major, presumably stressful, event. (b) The proportion of pregnant nonplacentophages that became placentophages rose over the course of pregnancy from a low not different from the unstressed virgin level, to a high, at the end of pregnancy, not different from the stressed virgin level. (c) The proportion of pregnant nonplacentophages that became placentophages during pregnancy did not even approach the parturitional level of proportion of placentophages (100%) by the last few hours before delivery. (d) Surgical termination of pregnancy did not elevate placentophagia beyond the level observed on that day of nonterminated pregnancy.

Kristal and Graber [4] found that 46.5% of a group of virgins obtained from a commercial breeder were placentophages, but only 4.1% of a group of laboratory-born virgins (daughters of rats obtained from the commercial breeder) were. They hypothesized that the proportion of placentophages in the purchased group was elevated by the stress they experienced during the shipping process. When tested as nonpregnant primiparae, purchased rats showed an incidence of placentophagia of 61.5%, and laboratory-born rats an incidence of 57.9%. These primiparae values were not significantly different from each other [4]. Although Kristal and Graber hypothesized that the increase in placentophagia from the virgin level to the nonpregnant primiparous level was due to experience with placenta acquired at the first delivery, it now seems more likely, considering the results of the present experiment, that pregnancy, perhaps as a stressful experience, and not experience with placenta at delivery, elevated the proportion of placentophages in the groups. The incidence of placentophagia in Kristal and Graber's laboratory-born group went from 4.1% as virgins to 57.9% as non-pregnant primiparae because stress elevated the tendency to eat placenta. Their purchased rats went from 46.5% placentophages as virgins to 61.5% as nonpregnant

primiparae, a relatively modest increase, because most of that group had already experienced stressful conditions before the first placentophagia test.

The gradual rise in placentophagia to stressed-virgin levels occurring during pregnancy, and the abrupt onset of universal placentophagia during delivery may actually reflect different processes. That which is produced by ongoing pregnancy, or more generally by presumably stressful events, seems irreversible. Once a nonpregnant female rat has eaten placenta, she remains placentophagic [3,4], whereas a large proportion of females observed to eat placenta during delivery, who would not eat placenta when they were virgins, return to being nonplacentophagic after

delivery [4]. It is possible, owing to the rapidity with which parturitional placentophagia develops, that at least that type of placentophagia is triggered by processes related to labor and delivery (e.g., sensory stimulation) rather than by factors related to pregnancy (i.e., hormones) that have been shown to facilitate pup-directed maternal behavior well before parturition. These data, therefore, add support to Kristal's hypothesis [3] that the onset of parturitional placentophagia, and perhaps to some extent parturitional pup-directed maternal behavior, may be produced by afferent information from the uterus during the early stages of labor and delivery.

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