Observing birth and placentophagia affects placentophagia but not maternal behavior of virgin rats

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To determine whether observing components of periparturitional behavior affects the manifestation of those behaviors in virgin rats, virgins selected for nonplacentophagia and for the absence of spontaneous maternal behavior toward pups were exposed to stimulus rats that were giving birth, eating donor placenta, or eating lab chow. During observations, subjects could either eat donor placenta or just see and smell it. The subjects were tested subsequently for placentophagia and for the rate of onset of pup-induced maternal behavior. The results indicated that: (1) access to placenta in the presence of other rats led to placentophagia; (2) when such placentophagia occurred in conjunction with exposure to other rats that were giving birth or eating donor placenta, the subjects became permanent placentophages (otherwise, the subjects reverted and did not eat on subsequent placentophagia tests); (3) none of the observation conditions, regardless of the availability of placenta during observation, affected the maternal sensitization latency. The results are discussed in terms of social facilitation, exposure learning, and desensitization to exteroceptive stimuli.

Most virgin female rats do not eat placenta when it is made available to them, yet virtually all parturient rats avidly consume placenta during delivery (Kristal, 1980; Kristal & Graber, 1976; Kristal, Peters, Franz, Whitney, Nishita, & Steuer, 1981). Stressful events, including pregnancy, elevate the likelihood of a rat’s eating placenta (placentophagia), although not to the level of behavior observed during parturition (Kristal et al., 1981). However, these results were obtained in laboratory situations, which typically use rats raised from weaning in relative isolation from other rats engaged in normal life activities. Birch (1956) noted that fact regarding investigations of maternal behavior, and his observation remains true for most maternal behavior research (Rosenblatt & Lehrman, 1963; Rosenblatt, Siegel, & Mayer, 1979; Sturman-Hulbe & Stone, 1929; Wiesner & Sheard, 1933). The normality of the maternal behavior exhibited by these relatively isolated rats was evidence, according to Birch (1956), of the irrelevance of social experience for the development of maternal behavior. He added, furthermore, that research results had shown “that rats are capable of learning little or nothing from observing the behavior of other rats” (p. 281).

Birch appears to have overstated the case. The presence of conspecifics has now been shown to affect behavior—both indirectly, as in the case of social facilitation (Dewsbury, 1978; Zajonc, 1965), and more importantly for this discussion, directly, as in the case of observational or exposure learning (Galef, 1976; Hall, 1980). The social transmission of information seems particularly important when it relates to feeding (Galef, 1977), and placentophagia is more an ingestive behavior that is characteristic of maternal females than it is a maternal behavior, in the sense of an infant-directed caretaking behavior. The social environment may also have an effect by providing an exposure to stimuli which, in itself, is sufficient to produce a gradual change in behavior toward those stimuli. Repeated exposure to donor placenta eventually causes virgins to stop avoiding it (Kristal, 1980), and constant exposure to pups induces appropriate maternal behavior in nulliparous rats (see Rosenblatt et al., 1979, for a review).

That placentophagia and pup-directed maternal behavior emerge apparently undiminished during parturition in socially isolated laboratory rats may be evidence of the unimportance of social experiences, as Birch (1956) suggested, but it is not evidence that social experience has no effect. The factors promoting the full constellation of periparturitional behaviors are probably more than supramaximal at parturition. Testing for the contribution of social experiences may require the use of a preparation in which the immediate, intense, hormonally based version of maternal behavior is absent.

The present study was designed to investigate the effect of observing parturition and placentophagia,
or placentophobia alone, on the subsequent response toward donor placenta of nonplacentophagic virgins. In addition, the effect of this experience on the induction rate of pup-directed maternal behavior (maternal sensitization latency) was assessed.

**METHOD**

**General Design**

Each rat, after we determined that she would neither eat placenta nor retrieve pups, was exposed to a rat giving birth and eating delivered placenta, to a nonpregnant rat eating donor placenta, or to a nonpregnant rat eating chow. During the observation period, the subject was presented with a donor placenta that she could see, smell, and eat (access condition), or see and smell, but not eat (no-access condition). Each stimulus rat, in the center compartment, was flanked by a pair of observers, one with access to placenta and the other without. The duration of exposure to nonpregnant stimulus rats was determined by the duration of delivery of the parturient rats. On the day after observing the stimulus rat, each subject was given a placentaphobic test (Test 1) and her response was recorded. Two days later, each rat began a period of constant exposure to foster pups (concaovas) to determine the rate of onset of pup-induced maternal behavior (maternal sensitization latency) (Cosnier & Couturier, 1966; Rosenblatt, 1967). Eight days later, whether or not the subject became maternal, she was given another single placentaphobic test (Test 2). The sets of six subjects exposed to the initial conditions together (the access rat and the no-access rat of each of the three sets) were always tested as a group on the same day.

**Subjects**

One hundred and ten virgin female Long-Evans rats, determined in pretests to be nonplacentophagic and not spontaneously maternal, served as subjects. All were about 75 days old and weighed about 225 g at their entry into the experiment. Thirty-eight of these rats were purchased at 7 weeks of age from a commercial supplier (Charles River Breeding Laboratories); the remaining 72 were born and raised in our laboratory and were the daughters of Long-Evans rats purchased from the same supplier. The rats from the two sources were distributed evenly across groups. An additional 55 Long-Evans females served as social stimuli for the subjects. When not in the experimental chamber, each rat was housed individually in a 45 × 19 × 25 cm hanging wire-mesh cage, which was fitted with a food hopper containing Charles River Rat/Mouse/Hamster Formula 3000, and with a water bottle. Food and water were available ad lib. The entire colony was on a 14:10 day:night cycle, with the day phase beginning at 0600 h (EST).

**Procedure**

**Placentaphobia Pretest**

When each rat was about 75 days old, we began daily examination of the cells in the vaginal smear to verify normal ovarian cyclicity. After the rat had undergone at least two consecutive normal estrous cycles, she was tested for her response to donor placenta. Placentaphobia tests were conducted during the third quarter of the light cycle (1200 to 1500 h). Each rat was allowed to habituate for 2 h to a wire-mesh cage in the testing room. Food was not available during the 2-h habituation period or during the test. Water was not available for the last 15 min of the habituation period or during the test. The test consisted of 15 min of exposure to a donor placenta presented in an unippable glass dish. If the rat ate it, she was designated a placentaphobe and returned to the colony. (These placentophobes served as stimulus rats during the observation sessions.) If not, she underwent the same procedure the next day and, if necessary, on the 3rd day. If she did not eat on the third exposure, she was designated a nonplacentophobe. Previous research has indicated that the response to placenta is dichotomous in virgins; either they eat it almost immediately or they appear to avoid it. Furthermore, eaters generally do so on the first exposure, and once a rat eats in this type of test, she reliably eats placenta afterward when it is made available (Kristal, 1980; Kristal & Graber, 1976). On the other hand, a small proportion of nonplacentophobes switch after stressful experience, so an additional single pretest (fourth placenta exposure), confirming nonplacentaphobia, was conducted and is described below.

Donor placentas were obtained surgically on Day 22 of pregnancy from CO2-killed Long-Evans multiparae. The placentas, placed three or four to a vial along with a few drops of physiological saline, were then immediately frozen at −20°C. When needed, the vial was rapidly thawed and the contents warmed to about 37°C for presentation.

**Spontaneous Retrieval Test**

A small proportion of virgin rats (extremely small in the Long-Evans strain) behave maternally toward pups as soon as the pups are presented. A few of these spontaneous retrievers distributed unevenly among the groups could bias the results, whereas a larger number might be expected to be distributed by chance more evenly and need not affect the results. Since the expected number was small and was likely to be distributed unevenly, we decided to identify and remove spontaneous retrievers from the pool of potential subjects.

Approximately 1 week after the placentaphobia pretest, each rat was placed in a wire-mesh-covered 26.7 × 52.1 × 30.5 cm glass chamber (10-gal aquarium) containing food, water, and 3 cm of coarse sawdust and was allowed to habituate to the new surroundings for 24 h. The aquaria were all kept in a separate test room. At about 0900 h, each rat was presented with four 3- to 8-day-old pups, which had been handled only with disposable plastic gloves. The presenter scattered the pups in the half of the aquarium that did not contain the rat's sleeping area. The rat was then observed for 15 min; if she retrieved the pups to a central site or even just carried them around the cage, she was designated a spontaneous retriever and was removed from the experiment. Only one spontaneous retriever was found.

**Fourth Placenta Exposure**

To avoid the possibility that the spontaneous retrieval test was stressful enough to cause some nonplacentaphobes to become placentaphobic, a single placenta test was conducted about 1 week after the spontaneous retrieval test. This test was actually a 4th day of placentaphobia pretest, conducted several days, rather than 1 day, after the third. If a rat ate placenta on this fourth exposure, she was removed from the experiment. None of the rats that had come this far in the pretesting had to be removed on the basis of the fourth placenta presentation.

**The Effects of Observing Stimulus Rats**

**Apparatus.** Three 10-gal aquaria were modified by inserting in each two perforated .25-in. Plexiglas panels, so that each aquarium was divided into three compartments, each measuring 26.7 cm long × 17.4 cm wide × 30.5 cm high. The panels were perforated with 65 .5-cm holes. Each of the three compartments was fitted with a water bottle and drinking tube; the two end compartments each contained a 4.5 × 5.4 × 3.5 cm Plexiglas feeder with a removable, perforated Plexiglas lid. The feeder was secured to the floor of the compartment. The aquaria were covered with lids of .5-in. mesh hardware cloth.

Each aquarium was housed in a sound-attenuating ventilated 41.9 × 92.1 × 40.0 cm box. Each box was fitted with a 4.5-in. exhaust fan, was lined with 1.25 cm of acoustic insulation, was lit by a timer-operated 7.5-W incandescent bulb, and had a one-way viewing port in the door.
Baseline effect. Two days after the fourth placenta exposure, 20 subjects were placed in observation compartments. Each pair of subjects flanked a normal, nonpregnant, non-food-deprived Long-Evans multipara. The stimulus rat had water available but not food. The trio remained in their compartments for 6.5 h (median duration of parturition, as determined in our laboratory). Ninety minutes before the end of the session, a placenta was put into the feeder of each subject's compartment. The feeder in one compartment had a perforated Plexiglas lid, enabling the subject to see and smell the placenta but not eat it (no-access group). The feeder in the other compartment had no lid, enabling the subject to eat the placenta (access group). At the end of the 6.5-h period, the rats were returned to their home cages and the test chambers were thoroughly cleaned.

The next day, each subject was given a standard placentaphagia test. Since only the access-group rats could have eaten the placenta in the observation compartment, the only criterion for placentaphagia was eating placenta on the test that occurred the following day. In this way, the proportion of nonplacentaphagophagic subjects that became placentaphagionic was known, and the proportion that ate during the observation period but did not eat on the placentaphagia test the next day, could be determined.

Observing parturient, placentaphagous, and chow-eating stimulus rats. Observation sessions were conducted on the day after the fourth placenta exposure. One subject was assigned randomly to each of the six conditions: (1) observing parturition and having access to placenta (part/access group); (2) observing parturition but not having access to placenta (part/no-access group); (3) observing placenta and having access to placenta (plac/access group); (4) observing placenta but not having access to placenta (plac/no-access group); (5) observing eating of chow and having access to placenta (chow/access group); and (6) observing eating of chow but not having access to placenta (chow/no-access group).

The access and no-access subjects were treated identically to corresponding subjects in the baseline situation, except for the determination of the duration of placenta exposure. In this phase of the study, all three stimulus conditions were run simultaneously. When the pregnant stimulus rat was in the afternoon of Day 22 of pregnancy, she was placed in the center compartment of one of the three chambers, and the other two stimulus rats and six subjects were placed in theirs. The pregnant stimulus rat and the six subjects were each given five pellets of chow. Twenty minutes after delivery of the first pup by the pregnant stimulus rat, we presented a donor placenta to each of the subjects and 18 donor placentas to the placentaphagic stimulus rat. The stimulus rats that would be observed eating chow had not been given food when moved to the observation compartment. They had, therefore, been food-deprived for 12-30 h. Twenty minutes after the beginning of delivery, when the placenta stimulus rat was given 18 placentas, the chow eater was given three full-size pellets of laboratory chow. At the completion of delivery, the subjects and placenta rats were returned to their home cages.

The next day, all six of the subjects in that battery were given a standard placentaphagia test (Test 1), as described above. To control for the amount of exposure to pups received by the subjects observing parturition, after Test 1 all subjects were returned to the same compartments they had occupied the previous day. This time, the four subjects that had observed rats without rat pups the previous day were exposed to a litter of 1- to 2-day-old rat pups, which was the same size as the litter delivered the day before. The litter (without an adult) was placed in the central compartment; duration of exposure to the litter was matched to the duration of parturition the previous day. The two subjects that had originally observed parturition were exposed to an empty central compartment during this phase of the experiment. After the exposure period, all subjects were returned to their home cages.

One day later, each subject was moved to a 10-gal aquarium containing 3 cm of coarse sawdust, a food hopper, and a water bottle. She was allowed to habituate to the cage for 24 h, after which she was given constant exposure to four 3- to 8-day-old foster pups, in a standard constant-procedure (Wiesner & Sheard, 1933). Pups were replaced at about 0000 h. Each time new pups were placed around the cage, the rat was observed for 15 min to determine whether she was maternal toward them. A second 15-min observation was conducted at about 2100 h. Criterion for maternal behavior was the presence of all of the following during the 15-min test: (1) retrieval of all four pups to a central site; (2) licking of the pups, particularly in the anogenital region; and (3) crouching over the pups (Rosenblatt, 1967). This convocation procedure was continued for 8 days, whether or not the subject became maternal.

On the day after the last day of pup-exposure, each subject was given another standard placentaphagia test (Test 2), which was conducted identically to Test 1. This overall procedure was repeated 15 times, until each of the six groups contained 15 subjects.

RESULTS

Baseline

The proportions of placentaphagophagic subjects found during the baseline assessment and during Tests 1 and 2 are presented in Table 1. Of the 10 rats in the baseline group that had had access to placenta during the observation session, three ate placenta the next day. Those three, and four others, had eaten the proffered placenta during the observation session, but only those three ate placenta during the placentaphagia test.

None of the 10 rats in the baseline group that did not have access to placenta during the observation session ate during the placentaphagia test on the next day. The difference in the proportion of placentaphagics between the access group (.30) and the no-access group (.00) was not significant (p = .11, Fisher exact probability test).

Test 1

In the access group, there were two more rats in each observation condition that ate placenta during the observation session than there were that ate in Test 1. This is similar to the situation that occurred among the baseline-group rats that had access to placenta. Using the proportion that ate during observation in the baseline group to compute the expected frequency of placentaphagophagic subjects during the observation session in Test 1 (expected frequency = 11), a chi-square analysis was performed on the observed frequencies (part/access = 12, plac/access = 10, chow/access = 7). The observed frequencies were not significantly different from the expected frequency [χ²(2) = 1.63, p > .05].

The proportion of placentaphagophagic subjects eating chow that had access to placenta (chow/access) was not significantly different from that of the baseline/access group (5/15 vs. 3/10; p = .61, Fisher exact probability test). The chow/no-access group was not significantly different from the baseline/no-access group (1/15 vs. 0/10;
Table 1
Effect of Observation on Induction of Placentophagia and on Maternal Sensitization Latency in Nonplacentophagic, Nonmaternal Virgin Rats

<table>
<thead>
<tr>
<th>Behavioral Test</th>
<th>Measure</th>
<th>N</th>
<th>Access to Placenta</th>
<th>Rat Only</th>
<th>Parturition</th>
<th>Placentophagia</th>
<th>Eating Chow</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline</td>
<td>Proportion ± SEP</td>
<td>10</td>
<td>Yes</td>
<td>.30 ± .145</td>
<td>.53 ± .129†</td>
<td>.33 ± .122</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>10</td>
<td>No</td>
<td>.00 ± .000</td>
<td>.07 ± .064</td>
<td>.07 ± .064</td>
<td></td>
</tr>
<tr>
<td>Test I</td>
<td>Proportion ± SEP</td>
<td>45</td>
<td>Yes*</td>
<td>.67 ± .122†</td>
<td>.50 ± .715</td>
<td>5.03 ± .562</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>45</td>
<td>No</td>
<td>.20 ± .103</td>
<td>.07 ± .064</td>
<td>.07 ± .064</td>
<td></td>
</tr>
<tr>
<td>Maternal Sensitization Latency</td>
<td>Mean ± SEM Days</td>
<td>45</td>
<td>Yes</td>
<td>4.90 ± .437</td>
<td>5.00 ± .488</td>
<td>6.37 ± .448</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>45</td>
<td>No</td>
<td>4.57 ± .452</td>
<td>5.00 ± .488</td>
<td>6.37 ± .448</td>
<td></td>
</tr>
<tr>
<td>Test II</td>
<td>Proportion ± SEP</td>
<td>45</td>
<td>Yes*</td>
<td>.73 ± .114†</td>
<td>.67 ± .122†</td>
<td>.33 ± .122</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>45</td>
<td>No</td>
<td>.13 ± .088</td>
<td>.07 ± .064</td>
<td>.07 ± .064</td>
<td></td>
</tr>
</tbody>
</table>

*Access groups significantly greater than no-access groups (p < .001).
†Significantly greater than value for chow/access group (p < .05).

p = .62, Fisher exact probability test). Therefore, observing a rat eating chow was as ineffective in altering the incidence of placenta as was observing a rat that was not eating.

A chi-square analysis was applied to the differences among the three observation conditions for the access group only (part/access, plac/access, and chow/access). Chi-square rather than ANOVA was used because in cases involving small groups and dichotomous data, the chi-square test is more powerful (Narula & Levy, 1977). The groups in the no-access condition were analyzed separately because the expected frequencies were too low for analysis with chi-square.

The analysis, using an expected frequency of 5 computed from the proportion of placenta groups produced in the baseline condition, indicated that there were significant differences among the observation conditions [$\chi^2(2) = 6.8$, p < .05]. Three subsequent two-way chi-square tests, which were adjusted for a joint alpha level with Bonferroni's procedure (Myers, 1972), indicated that the proportions of placenta groups in the part/access and plac/access groups were significantly greater than that in the chow/access group, but were not significantly different from each other (joint α = .05).

The observation conditions on the no-access group were not significantly different from each other (3/15 vs. 1/15; p = .30, Fisher exact probability test).

A 3 x 2 ANOVA, comparing three levels of observation and two levels of access to placenta, was used solely to determine whether there was any effect of the interaction between the two variables. The results indicated that there was no significant effect of this interaction on placenta in Test 1 [$F(2,84) < 1.0]$.

The difference between the access and no-access conditions was analyzed by pooling the data across observation groups and computing the standard error of the difference between the two resulting proportions, 23/45 and 5/45 (Dixon & Massey, 1969). The proportion of placenta groups in the access group was significantly greater than that in the no-access group ($d_p/n = .089, z = 4.09, p < .001$).

Maternal Sensitization Latency
The mean latencies (± SEM), in days, for the onset of maternal behavior during the concavation procedure (maternal sensitization latencies) for the groups, are presented in Table 1.

An ANOVA was used to determine whether there was any effect of the three types of observation or of the two access conditions on the rate of onset of pup-induced maternal behavior. The results of the analysis indicated that there was no significant effect of observation condition [$F(2,84) = 1.80$, p > .05], of access condition [$F(1,84) < 1.0$], or of the interaction between those two main effects [$F(2,84) = 1.40$, p > .05].

Test 2
The Test 2 data consisted of the results of a placenta test conducted after the concavation procedure.

An ANOVA, which compared two tests (repeated measures), three observation conditions, and two access conditions, indicated that there was no significant difference between Test 1 and Test 2 [$F(1,84) = 1.04$, p > .05]. The interaction between the access variable and the test variable was significant [$F(1,84) = 4.15$, p = .04]. This was because one placenta group in the part/access group and two in the plac/access group became placenta after the maternal sensitization phase of the experiment (see Table 1). Also, one rat in the part/no-access group that had eaten in Test 1 did not eat in Test 2. It is impossible
to determine at this point whether those switches were a result of the procedure that occurred in the interval between Tests 1 and 2 or whether those rats belonged to the extremely small group whose behavior toward placenta is inconsistent (Kristal & Graber, 1976).

**DISCUSSION**

Virgin rats were given the opportunity to observe rats that were giving birth, rats that were eating donor placenta, or rats that were eating chow. During the observation session, the observer rats could smell, see, and eat donor placenta or merely smell and see it. The baseline response to the test chamber and to the presence of other rats was also determined. The results indicated that: (1) having other rats present, and being in the chamber, when placenta was available produced a high incidence of placentophagia during the observation session; (2) when placenta was available during the observation session, observing parturition (and therefore placentophagia) or placentophagia alone, produced significantly more conversions of nonplacentophages to placentophages than did observing a rat eating chow; (3) the number of conversions produced by observing a rat eating chow was not greater than that produced by the baseline condition (rat only); (4) never observing a rat giving birth nor observing a rat eating donor placenta, whether or not placenta was available for the observer rat to eat, was effective in altering the rate at which the observer rat was induced to behave maternally by constant exposure to pups; and (5) concaveation did not produce a significant increase in the proportion of placentophages beyond that produced by the observation procedure.

The effects of the procedure on placentophagia could be interpreted as a result of one or more of three main processes: social facilitation—an increase in some characteristic of a response (in this case, the likelihood of a response) because of the presence of other individuals (Dewsbury, 1978; Zajonc, 1965); observational or exposure learning—the social transmission of an acquired behavior (Galef, 1976, 1977; Hall, 1980); or sensitization or desensitization to a stimulus, brought about by constant or repeated exposure to the stimulus (Kristal, 1980; Rosenblatt et al., 1979). Although the present study was not undertaken specifically to select among these processes, but rather to demonstrate the existence of an effect, some conclusions about the three processes can be drawn.

None of the groups in the no-access condition, including the baseline group, were susceptible to the effects of social facilitation, since they could not engage in placentophagia when other rats were present. (We assumed that the effect of the novel chamber was minimal, since placentophagia tests are often conducted in novel environments in our laboratory with little or no effect.) Furthermore, since the baseline group observed stimulus rats that were not eating anything, exposure learning was probably not taking place. However, placenta was present, and the sight and smell of it could have been having an effect on the subject, but the results indicated otherwise; no baseline-group nonplacentophages were converted into placentophages by this experience. In the other no-access groups, the combination of the presence of placenta (for desensitization) and the stimuli for exposure learning of placentophagia was insufficient to convert a significant proportion of nonplacentophages to placentophages.

The baseline/access group had the stimuli for desensitization (the presence of placenta) and the stimulus for social facilitation (an audience), but not the stimulus for exposure learning. Although social facilitation was sufficient to cause a high proportion of rats to eat during the observation session, desensitization coupled with eating produced by social facilitation were not sufficient to cause those eaters to become placentophages permanently; most did not eat on the subsequent placentophagia test. Access-group rats exposed to stimulus rats that were eating something or giving birth were receiving the stimuli for desensitization (all three groups), for social facilitation (all three groups), and for exposure learning of placentophagia (part/access and plac/access groups only). Since the part/access and plac/access groups showed the greatest increase in proportion of placentophages after the observation session, it seems that exposure learning does have an effect when the behavior observed can be practiced during the exposure, and that some behaviors (e.g., placentophagia in nonplacentophagic virgin rats) require social facilitation for initiation. That socially facilitated rats eat placenta but do not necessarily become permanently placentophagic may partly explain why nonplacentophages eat placenta during parturition, yet unlike virgin nonplacentophages that become placentophagic after a stressful experience, do not necessarily eat placenta when tested subsequently (Kristal, 1980; Kristal & Graber, 1976; Kristal et al., 1981).

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