

# Placentophagia in Rats is Modifiable by Taste Aversion Conditioning<sup>1</sup>

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ENGWALL, D. B. AND M. B. KRISTAL. *Placentophagia in rats is modifiable by taste aversion conditioning*. *PHYSIOL. BEHAV.* 18(3) 495–502, 1977. — An aversion to placenta was conditioned by pairing ingestion with LiCl-induced illness, in virgins, in nonpregnant primipara, and in primipara during the first parturition. Persistence of the aversion was assessed at the subsequent parturition, immediately after the subsequent parturition, and two weeks after the subsequent parturition. The results indicated that (a) female rats can learn an aversion to placenta, (b) the aversion was expressed during parturition, (c) previous parturitional experience reduced retention of the aversion, but not acquisition, (d) rats can distinguish between their own normally delivered placenta and donor placenta, and (e) an aversion to placenta at parturition did not appear to have a major effect on pup care.

Placentophagia    Taste aversion conditioning    Maternal behavior    Rats    Lithium chloride

PREVIOUS studies have demonstrated the difficulty in distinguishing the maternal behavior of the experienced, multiparous female rat from her inexperienced, primiparous counterpart [8]. The importance of prior parturitional experience can, however, be observed under atypical breeding conditions brought about by ovariectomy [9] and olfactory bulbectomy [1]. The experience of raising the first litter buffers the parturient female against the disruptive effects of the atypical physiological conditions.

The present experiments were undertaken, in part, to investigate the effects of experience on the specific maternal behavior of placentophagia, the consumption of the afterbirth by the parturient female. Placentophagia usually occurs only under the peculiar hormonal and behavioral conditions of parturition, and can be considered both as an ingestive and a maternal behavior.

In a study of the relationship between placentophagia and homeostatic feeding, Kristal [4] produced lesions of the lateral hypothalamus (LH) in pregnant and nonpregnant rats. Despite the aphagia demonstrated by all the animals with lesions, multiparae continued to eat placenta at parturition, whereas primiparae did not. Kristal also found that virgins did not ingest donor placenta after receiving lesions, whereas nonpregnant multiparae continued to eat placenta after becoming otherwise aphagic. Kristal concluded that prior parturitional experience with placenta, and not pregnancy per se, was the critical factor influencing the occurrence of placentophagia after LH lesions.

A certain proportion of nonpregnant rats and mice will eat placenta that has been obtained from donor females [4,

5, 6, 7,]. Recent studies have shown increases in placentophagia as a function of parity in Long-Evans rats [6]. The proportion of females exhibiting placentophagia in the nonpregnant state as related to parity was as follows: nulliparous, 0.47; primiparous, 0.62; multiparous, 0.76. Thus the influences of parity on placentophagia can be seen in both the parturient and the nonpregnant conditions.

In discussing the motivational factors that might inhibit placentophagia in virgin female mice, Kristal and Eleftheriou [5] suggested that neophobia or aversion might be competing with the initiation of placentophagia. The competing avoidance response appeared to be overridden by the behavioral and/or hormonal events which surround parturition, since nearly every female rodent consumes placenta during her first normal parturition. The subsequent ingestive response to placenta was altered as a result of parturitional experience with placenta.

Since taste aversion conditioning (hereafter, for the sake of brevity, TAC) has been used to investigate the development of ingestive preferences [2], the application of TAC techniques seemed a logical approach to the study of placentophagia. TAC involves the pairing of a novel taste with gastrointestinal distress brought about by the administration of a toxic agent. For the present experiments, the consumption of placenta was paired with the administration of lithium chloride (LiCl), a very effective malaise-inducing agent [10].

Novelty has been found to be of great importance in assuring the effectiveness of TAC. Rats learn aversions to novel substances much more readily than to familiar ones

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[11,12]. The relative difficulty seen when inducing aversions to non-novel substances has been attributed to the learned safety hypothesis of Kalat and Rozin [3]. If an animal ingests a novel substance and suffers no subsequent malaise, the animal learns that the substance is safe to consume. The strength of this learned safety increases as a function of the exposure to the ingested substance prior to TAC.

If placentophagia can be considered a normal ingestive behavior, then its incidence should be modifiable by the application of TAC. Animals with little previous safe exposure to placenta should avoid the substance after it has been paired with toxicosis. On the other hand, if placentophagia is controlled by mechanisms other than those controlling feeding, or if gustatory cues are unimportant, or if placenta is a prepotent or unique stimulus, TAC should be ineffective in modifying placentophagia. The present research sought to determine the extent to which the incidence of placentophagia could be altered by the taste aversion techniques. The questions asked were: (a) is placentophagia during a nonpregnant state susceptible to TAC? (b) Could an aversion acquired during a nonpregnant condition modify placentophagia during a subsequent parturition? (c) Could an aversion be conditioned with equal effectiveness in both nonpregnant and parturient conditions? (d) Would prior parturitional experience with placenta alter the induction and expression of a taste aversion to placenta?

#### PLACENTOPHAGIA PRETEST

All animals were given a placentophagia pretest to insure that they all belonged to the population of animals that exhibit placentophagia during a nonpregnant state [6]. Since it had been observed that animals that eat placenta during the nonpregnant state continue to do so reliably [6], any decrease in the response to placenta could then be attributed to the expression of a taste aversion to placenta.

#### METHOD

##### *Animals*

One hundred eighty-three Long-Evans female rats (Charles River Breeding Laboratories) were used in the placentophagia pretest. The females were approximately 60 to 75 days old upon their arrival in the laboratory and were all virgins with no known previous exposure to placenta. Females were housed in 18 × 18 × 24 cm wire mesh cages and maintained on a 14 hr on; 10 hr off light cycle with the on-phase beginning at 6:00 a.m. (EST). Charles River Rat/Mouse/Hamster Formula and water were available ad lib, except as indicated below.

##### *Procedure*

After 1 week of colony residence, daily vaginal smears were obtained until normal estrous cyclicity was confirmed. Each female was then given the placentophagia pretest [6]. At 10:00 a.m. on the first day of testing, females were moved to a quiet, well lighted testing room, and placed in a 16 × 16 × 48 cm clear plastic cage, with a wire mesh grid covering the floor (test cage). Water was available, but no food. Two hr later, the water was removed. Fifteen min later, each female received a 15 min exposure to donor placenta. Donor placenta for the pretest and Experiments 1 and 2 was removed surgically from CO<sub>2</sub>-killed females on

Day 21 of pregnancy, and frozen in 6 ml glass vials along with a few drops of physiological saline. Immediately prior to presentation, the vials were warmed in a hot water bath, to about 37°C. Each female was given 2 whole placentas in a small specimen dish. After the 15 min exposure period and after the female's response to placenta was recorded, vaginal smears were taken and the females returned to wire mesh cages. The testing procedure was terminated when a female ate placenta, or after 3 successive daily tests. Only females that ate placenta during the pretest were used for subsequent experiments.

#### EXPERIMENT 1

Experiment 1 was designed to examine whether a conditioned taste aversion to placenta, produced in virgin females, would be expressed as an aversion to placenta during the first parturition. Since the females in Experiment 1 had no parturitional experience at the onset of conditioning, their previous experience with placenta was limited to the exposure obtained during the pretest.

#### METHOD

##### *Animals*

The animals were 20 virgin Long-Evans rats (90–100 days old) that had exhibited placentophagia in the pretest.

##### *Procedure*

*Taste aversion conditioning.* Females were randomly assigned to 1 of 2 groups: Group C (contingent) and Group CP (noncontingent-pregnant). One week after the pretest, females in Group C (n = 15) were subjected to TAC. On Day 1 of the conditioning procedure, females were moved to the testing room and placed in plastic cages with wire mesh grid inserts covering the cage floors. Animals in Group C were food deprived for 2¼ hr and water deprived for 15 min prior to the presentation of donor placenta. Immediately after 15 min of exposure to placenta, those females that consumed placenta were given an injection of 0.15 M LiCl solution (20 ml/kg body weight, i.p.). Vaginal smears were then obtained and the females were returned to their home cages. Food and water were removed for a 2 hr period following the injection to minimize any possible interfering effects of ingestion while malaise developed. Beginning on the next day (Day 2), vaginal smears were obtained daily to assess the effects of LiCl on estrous cyclicity. The females were then tested for an acquired aversion to placenta for 3 consecutive days (Days 4, 5 and 6). The criterion was abstention from placentophagia on all 3 days. Females failing to reach criterion after the first pairing of LiCl-induced illness with placentophagia were given additional pairings until criterion was reached. As a control for the effects of LiCl injections per se, Group CP (n = 5) were presented with empty dishes during the conditioning and test trials. After a 15 min exposure to the empty dish, the females were given an injection of LiCl. Two such injections of LiCl were administered in a temporal sequence which approximated the procedure used with Group C.

*Mating.* Upon reaching criterion, Group C was divided into 2 subgroups: Subgroup CP (contingent-pregnant, n = 7) and Subgroup C $\bar{P}$  (contingent-nonpregnant, n = 8). Within 1 week after reaching criterion, each female in Subgroup CP and Group C $\bar{P}$  (but not Subgroup C $\bar{P}$ ) was

placed with a breeder male. Vaginal smears were obtained every morning until sperm in the vaginal smear or a vaginal plug was observed (Day 1 of pregnancy).

*Observations at the first parturition.* Pregnant females of Subgroup CP and Group  $\bar{CP}$  were moved to the testing room on the afternoon prior to the expected date of parturition (Day 22). (The unequal group sizes are accounted for by the removal, at this point, of females failing to become pregnant.) Each female was placed in a test cage; food, water, and a paper towel for nest-building were provided. The testing room was maintained on a light-dark cycle synchronized with that of the colony room. A 40 W red light bulb provided illumination during the dark period. Beginning at 9:00 the next morning, each pregnant female was checked at 30 min intervals until she began to deliver. The entire parturition was observed and notes were taken on perinatal behavior with special emphasis on placentophagia.

Thirty min after delivery of the last pup, the litter was removed from the cage. Unattached placentas were also removed and counted. The litter was inspected to determine whether the pups had been cleaned and the placentas detached. The litter was placed under a 75W white incandescent bulb to provide warmth. To assess the expression of an aversion to placenta in the parturition tests only, a placentophagia index was computed by dividing the number of consumed placentas by the total number of placentas delivered.

Immediately after the removal of the litter, the female was presented, for 15 min, with 4 donor placentas in a specimen dish. Then, the litter was reintroduced into the cage and the female's latency to retrieve all pups to the nest site was recorded. The mesh grid was then removed, and coarse sawdust placed in the cage for nestbuilding. The cage containing the female and her litter was moved to a metal rack in the testing room.

*Observations of maternal behavior at 2 days postpartum.* Two days after parturition, the female was tested for several general indices of maternal behavior: the presence of a well-built nest, the number of surviving pups, and the arrangement of the litter. The litter was then scattered around the cage floor and the female's latency to retrieve the litter to the nest site was recorded: if the female did not retrieve her litter during a 15 min test period, she was

recorded as not having exhibited retrieval. The litter was then removed and the female returned to her home cage.

*Placentophagia test at 2 weeks postpartum.* Another measure of placentophagia of donor placenta was taken 2 weeks after the date of parturition (39 days after conditioning) for females in Group  $\bar{CP}$  and Subgroup CP.

*Testing procedure for nonpregnant females.* Females in Subgroup  $\bar{CP}$  were given an opportunity to consume donor placenta 25 days after reaching criterion in TAC (comparable to the interval prior to the parturition test for Subgroup CP) and again at 39 days after reaching criterion (comparable to the interval between conditioning and the postpartum test for Subgroup CP).

## RESULTS

### *Taste Aversion Conditioning*

The majority of animals receiving TAC (Subgroups CP and  $\bar{CP}$ ) required 2 pairings of placenta with LiCl-induced illness to reach criterion. The mean number of pairings-to-criterion for each group is presented in Table 1. Most animals exhibited diarrhea and general inactivity following the injection of LiCl, but no long-term effects such as weight loss were noted. All females continued to show ovarian cyclicity as verified by vaginal smears obtained throughout the conditioning procedure.

### *Observations of Placentophagia at the First Parturition*

The 2 measures of placentophagia (ingestion of delivered placenta during parturition, and of donor placenta immediately after parturition) were combined to provide an indication of the female's response to placenta at parturition. To be scored as exhibiting an aversion to placenta at parturition, an animal had to manifest both a decrement in the consumption of her own placenta during parturition and the absence of ingestion of donor placenta presented immediately after parturition.

All the females that had been treated with LiCl unpaired with placenta (Group  $\bar{CP}$ ) continued to exhibit placentophagia during, and immediately after, parturition. The conditioned females (Subgroup CP) exhibited an aversion to placenta at parturition: 6 of the 7 animals in Subgroup CP showed a decrement in placentophagia during parturi-

TABLE 1

DESIGN AND RESULTS OF EXPERIMENT 1: TAC TO PLACENTA INDUCED IN NULLIPARAE  
(PROPORTION OF GROUP EXHIBITING PLACENTOPHAGIA)

|                            | CP          | Group<br>$\bar{CP}$ | $\bar{CP}$    |
|----------------------------|-------------|---------------------|---------------|
| N*                         | 7           | 8                   | 5             |
| Treatment                  | TAC         | TAC                 | LiCl-unpaired |
| no. of administrations     | 2.12 ± 0.23 | 2.12 ± 0.12         | 2.00 ± 0.00   |
| Mating                     | YES         | NO                  | YES           |
| Proportion Placentophages: |             |                     |               |
| during parturition         | 0.14        |                     | 1.00          |
| immediately after          | 0.00        |                     | 1.00          |
| control interval           |             | 0.13                |               |
| 2-wk postpartum interval   | 1.00        | 0.25                | 1.00          |

\*All animals determined to be placentophages in a pretest.

tion and the complete absence of placentophagia to donor placenta just after parturition (see Table 1). Placentophagia during parturition was completely eliminated in 2 animals (placentophagia index of 0.00). Placentophagia scores for the 6 animals ranged from 0.00 to 0.75, with a mean score of  $0.26 \pm 0.12$ . When Group CP was compared with the group of females given TAC (Subgroup CP), the difference in the incidence of placentophagia at parturition was significant ( $p = 0.008$ , by the Fisher exact probability test).

Females in Subgroup CP that exhibited an aversion spent abnormally long periods of time devoted to attempts at consuming placenta. While the consumption of placenta in CP and in normal females was usually completed within 1 min after delivery, females exhibiting the aversion would pick up the placenta, chew vigorously on the attached umbilicus for several minutes only to drop the placenta, eventually, on the cage floor. Females would often return to the placenta after a few minutes and attempt to consume it once again. During the time devoted to attempts at placentophagia, the female spent very little time caring for her litter. Although the females continued to clean the fetal membranes from their pups, the pups were often observed scattered about the cage with the umbilicus and placenta still attached.

#### *Observation of Maternal Behavior*

When observed at 2 days postpartum, all the females in both pregnant groups (Subgroup CP and Group CP) built nests, nursed their pups and kept their litter in a single pile within the nest. Two animals in each of the 2 groups failed to retrieve their pups during the 15 min retrieval test. In the group that had exhibited an aversion to placenta at parturition (Subgroup CP), 4 of the 7 females lost a small number of pups from the litter size recorded at parturition, the mean number of pups lost being  $2.0 \pm 0.7$ . Although there was no pup loss observed in the noncontingent LiCl treatment group (Group CP), the 2 groups were not significantly different ( $p = 0.07$ , Fisher test).

#### *Two week Postpartum Test*

During this test (39 days after TAC), all females in both groups ate placenta. The response of the conditioned group (CP) was of particular interest. Whereas only 1 of the 7 animals in this subgroup had eaten placenta at parturition, all exhibited placentophagia during the 2 wk postpartum test. This change in response was found to be significant ( $p = 0.02$ ; McNemar test).

#### *Response of Nonpregnant Females (CP)*

In the test given to these animals after the control interval (25 days after TAC), only 1 of the 7 females exhibited placentophagia: this was very similar to the response of the pregnant group (Subgroup CP) at parturition.

A difference in the expression of the aversion in the pregnant and nonpregnant groups did appear, however, when both groups were tested on donor placenta 39 days after reaching criterion. Whereas 6 of 8 females in the nonpregnant group (Subgroup CP) were still exhibiting an aversion to placenta at 39 days, none of the females in the group that had completed their first parturition (Subgroup CP) exhibited an aversion to placenta at this time. This difference was statistically significant ( $p = 0.006$ , Fisher test).

In summary, females conditioned and tested as virgins (Subgroup CP) expressed an aversion to placenta that was observable 25 and 39 days after induction. Females conditioned as virgins and tested at parturition (Subgroup CP), expressed the aversion to placenta at the first parturition but not when tested 2 weeks after parturition. Females given LiCl unpaired with placenta as virgins (Group CP), did not express an aversion to placenta either at parturition or 2 weeks after parturition.

## EXPERIMENT 2

Experiment 2 was designed to assess the effects of TAC to placenta induced during the nonpregnant state following the first pregnancy (prior parturition experience) on the incidence of placentophagia during the second parturition. Before the administration of TAC, females in Experiment 2 completed a normal parturition which included the consumption of placenta.

### METHOD

#### *Animals*

Animals for Experiment 2 were 15 female rats that had exhibited placentophagia during the pretest.

#### *Procedure*

*Mating and observations at the first parturition.* Within 1 week after the pretest, each virgin female was bred using procedures described in Experiment 1. On the afternoon of Day 21 of gestation, females were moved to the testing room and placed in test cages. They were allowed to complete a normal parturition, including the consumption of placenta. Within 8 hr after delivery of the last pup, the litter was removed and the female returned to her home cage.

*Taste aversion conditioning.* One week after parturition, the taking of vaginal smears to confirm estrous cyclicity was begun. Two weeks after parturition, TAC was administered as in Experiment 1.

*Mating and observations at the second parturition.* Upon reaching the criterion for taste aversion, the sample was divided into 2 groups: Group P (pregnant,  $n = 7$ ), and Group P̄ (nonpregnant,  $n = 8$ ). The 2 groups were balanced for the number of pairings to reach criterion as in Experiment 1. Females in Group P were mated within 1 week after reaching criterion. During the second parturition, observation of placentophagia and other perinatal maternal behaviors was conducted as in Experiment 1. Females in Group P̄ were not mated.

*Observation of maternal behavior and 2 week postpartum test.* Testing procedures were as described in Experiment 1.

*Testing procedures for nonpregnant females.* Females in Group P̄ were given a single exposure to donor placenta 30 days after reaching criterion in TAC. The second control interval test was administered as in Experiment 1 and took place 44 days after reaching criterion.

## RESULTS

#### *Taste Aversion Conditioning*

Two weeks after the first parturition, all females having resumed normal estrous cyclicity, females in Experiment 2 were given TAC. The mean number of pairings-to-criterion

TABLE 2

DESIGN AND RESULTS OF EXPERIMENT 2: TAC TO PLACENTA INDUCED IN NONPREGNANT PRIMIPARAE (PROPORTION OF GROUP EXHIBITING PLACENTOPHAGIA)

|   | P           | Group<br>P  |
|---|-------------|-------------|
| N*  | 7           | 8           |
| Mating  | YES         | YES         |
| Proportion Placentophages:<br>during parturition I  | 1.00        | 1.00        |
| Treatment   | TAC         | TAC         |
| no. of administrations                              | 2.14 ± 0.81 | 2.25 ± 0.25 |
| Mating  | YES         | NO          |
| Proportion Placentophages:<br>during parturition II | 0.71        |             |
| immediately after                                   | 0.00        |             |
| control interval                                    |             | 0.37        |
| 2-wk postpartum interval                            | 0.43        | 0.88        |

\*All animals determined to be placentophages in a pretest.

for each group is presented in Table 2. The difference between the mean number of pairings in Experiments 1 and 2 was not significant,  $t(28) = 0.34$ ,  $p > 0.10$ . The prior experience with placenta of animals in Experiment 2 did not result in a greater number of pairings to reach criterion.

#### Observations of Placentophagia at the Second Parturition

At the second parturition, 5 of the 7 animals in the pregnant group (Group P) exhibited normal placentophagia to their own delivered placenta. The remaining 2 animals showed an aversion to their own placenta as indicated by placentophagia scores of 0.46 and 0.33, with a mean score of  $0.40 \pm 0.06$ . Considered as a whole, Group P did not show an aversion to their own placenta during parturition. When parturient females were compared with their pretest levels of placentophagia, females in Group P did not show a significant change in their response to placenta at parturition (see Table 2). Although 5 of the 7 parturient females had eaten their own delivered placenta during parturition, none ate donor placenta presented immediately after parturition ( $p = 0.03$ , McNemar test).

Although comparisons between the primiparae in Experiment 2 and the nulliparae in Experiment 1 revealed no differences in the number of pairings to reach criterion, differences were observed in the expression of the aversion to naturally delivered placenta during parturition. Females that had had prior parturitional experience were significantly less likely to exhibit an aversion to their own placenta during a subsequent parturition (Group P; 5 of 7 ate) than were females that had no prior parturitional experience (Group CP; 1 of 7 ate) ( $p = 0.05$ , Fisher test).

#### Observations of Maternal Behavior

During the perinatal period, 1 female (in Group P) that exhibited an aversion to placenta failed to clean a large number of pups in her litter. When observed at 2 days postpartum, the female had lost 9 pups from her litter. Four other females each lost 1 pup. All the females retrieved their pups, both after parturition and at 2 days postpartum. All of the females built nests, nursed their pups and kept their litters in a single pile within the nest.

#### Two week Postpartum Test

Whereas 5 of the 7 females in Group P had eaten placenta during parturition, only 3 ate donor placenta at 2 weeks postpartum. All 3 had eaten at parturition but not in the immediately-postpartum test. The number of animals eating placenta during the 2 week postpartum test was not significantly different from the number eating their own placentas during parturition ( $p = 0.31$ , McNemar test) nor, partly due to the small group size, from the number eating during the pretest ( $p = 0.06$ , McNemar test), nor from the number eating during the immediately-postpartum test ( $p = 0.13$ , McNemar test).

To summarize the effects of TAC on placentophagia in the pregnant experienced group, at parturition, immediately after parturition, and at 2 weeks postpartum, the only aversion observed was to donor placenta presented immediately after parturition.

#### Response of Nonpregnant Females

Three of the 8 animals in the experienced nonpregnant group (Group P) ate placenta in the test after the first control interval (30 days after conditioning). This proportion is significantly lower than pretest level ( $p = 0.03$ , McNemar test). Note that placentophages are expected to eat during retests, e.g., Group CP in Experiment 1.

The results of the first nonpregnant-interval test for Subgroup CP (Exp. 1) and Group P (Exp. 2) were compared. Seven of the 8 inexperienced females (Subgroup CP) exhibited the aversion as compared with 5 of the 8 experienced females (Group P). Although the trend was in the expected direction, the difference was not significant since the number of animals tested was small ( $p = 0.28$ , Fisher test). Thus the effects of prior parturitional experience did not appear to alter the expression of an aversion to placenta when the animals were conditioned and tested in the nonpregnant state.

When tested after the second control interval (44 days after conditioning), 7 of the 8 animals in the experienced, nonpregnant group (Group P) exhibited placentophagia. Although this proportion is larger than that observed after the first control interval (3/8), due to the small group size, this increase was not significant ( $p = 0.06$ , McNemar test). Group P did not differ significantly from Group P on the 44 day test ( $p = 0.26$ , Fisher test).

In summary, the nonpregnant group (Group P) demonstrated an aversion to placenta when tested at the gestational interval (30 days), but not after 44 days. Females in the pregnant group (Group P) did not show an aversion to their own placenta during parturition, but they refused to eat donor placenta presented just after parturition. As in the nonpregnant group, most of the pregnant females ate donor placenta after 44 days. Although the 2 groups did demonstrate an aversion to placenta at different times, the groups were not significantly different from each other during any of the tests.

### EXPERIMENT 3

Experiment 3 was designed to examine the effects of TAC induced during the first parturition on the incidence of placentophagia at the second parturition. Females in Experiment 3 received conditioning immediately after the completion of parturition, whereas females in Experiments 1 and 2 had been conditioned during the nonpregnant, cycling state.

## METHOD

*Animals*

Subjects were 25 virgin female rats that had exhibited placentophagia during the pretest.

*Procedure*

*Mating and procedure at the first parturition.* Females were assigned to 1 of 2 groups, Group L (LiCl treatment), or Group SP (Saline control-pregnant). Both groups were mated within 1 week after completion of the pretest. Breeding procedures were as in Experiment 1.

Females were moved to test cages on the afternoon prior to the date of expected parturition. When the female began to deliver, food and water were removed from the cage. As the females delivered each placenta, the placenta was grasped with a pair of large forceps and removed from the cage. If the pup was detached from the placenta before the delivery of the placenta, the placenta alone was collected. If the pup and the placenta were delivered as an intact unit, both were removed from the cage. The pup was returned after the placenta had been detached from the pup by the experimenter.

The collected term placentas were immediately placed in a 23 ml glass vial which contained a few drops of saline, and were maintained at 0°C in an ice water bath throughout the parturitional sequence. Thirty min after the delivery of the last pup, 4 placentas were removed from the collection vial and warmed to about 37°C. The remaining placentas were transferred to 6 ml vials and frozen with a few drops of saline for further use.

The litter was removed from the puerpera's cage and placed under a 75 W, white incandescent bulb. The warmed placentas were transferred to a specimen dish and placed in the female's cage. As soon as the female consumed all the presented placentas, females in Group L were injected with a 0.15 M LiCl solution (20 ml/kg body weight, IP). Females of the Group SP were injected with a comparable volume of isotonic saline. All injections were completed within 15 min after the consumption of the placenta. Females (but not the litters) were returned to their home cages. Food and water were removed for 2 hr following the injection of LiCl or saline.

*Mating and observations at the second parturition.* Group L was divided into 2 subgroups, Subgroup LP (pregnant) and Subgroup LP̄ (not pregnant). Because of the difficulties entailed in collecting placenta from parturient females, it was impossible to completely eliminate placentophagia during parturition in every female. Females were assigned to the 2 subgroups (n = 9 for each) on the basis of the number of placentas eaten during the first parturition; both subgroups contained females that had consumed small ( $\leq 2$ ) and large ( $> 2$ ) numbers of placentas during parturition. The mean number of placentas consumed during the first parturition for Subgroups LP and LP̄ were  $2.11 \pm 0.68$  and  $3.33 \pm 0.90$ , respectively. The mean number of placentas consumed by the saline control group, Group SP, was  $4.17 \pm 0.79$ . A one-way ANOVA revealed that these group values were not significantly different,  $F(2,21) = 1.54, p > 0.10$ .

Females of Subgroup LP and Group SP were allowed 1 week after parturition before the vaginal smear procedure was begun. Females of the 2 pregnant groups were then mated using procedures described in Experiment 1.

Thirty min after the delivery of the last pup, females in Group SP and Subgroup LP were presented with 4 term placentas in a specimen dish. The term placenta was that which had been collected during the first parturition. Otherwise, the procedures for parturitional observations were identical to those used in Experiment 1.

*Observations of maternal behavior and 2 week postpartum test.* Both procedures were conducted as in Experiment 1 except that term placenta, rather than surgically obtained donor placenta, was used.

*Testing procedures for nonpregnant females.* Females in Subgroup LP̄ were given a single exposure to term placenta 38 days after parturition. The first control interval approximated the interval between the first and second parturitions of females in Subgroup LP.

Term placenta was also used for test after the second control interval; this test was administered to Subgroup LP̄ 52 days after the first parturition.

## RESULTS

*Observations at the First Parturition*

The term placenta presented immediately after the first parturition was completely consumed by all females in the 2 groups given TAC (Subgroups LP and LP̄). In the group that was to receive a saline injection (Group SP), 1 female failed to consume the presented term placenta; after elimination of this animal from the experiment, Group SP contained 6 animals.

TABLE 3

DESIGN AND RESULTS OF EXPERIMENT 3: TAC TO PLACENTA INDUCED AT FIRST PARTURITION (PROPORTION OF GROUP EXHIBITING PLACENTOPHAGIA)

|                            | LP   | Group LP̄ | SP     |
|----------------------------|------|-----------|--------|
| N*                         | 9    | 9         | 6      |
| Mating                     | YES  | YES       | YES    |
| Treatment (parturition I)† | TAC  | TAC       | Saline |
| Mating                     | YES  | NO        | YES    |
| Proportion Placentophages: |      |           |        |
| during parturition II      | 0.44 |           | 1.00   |
| immediately after          | 0.11 |           | 0.83   |
| control interval           |      | 1.00      |        |
| 2-wk postpartum interval   | 1.00 | 0.89      | 1.00   |

\*All animals determined to be placentophages in a pretest.

†Only 1 pairing was given in all cases.

*Observations of Placentophagia at the Second Parturition*

Five of the 9 animals in Subgroup LP (pregnant, LiCl treated) showed a decrement in the consumption of their own placenta during parturition (placentophagia scores ranged from 0.77 to 0.13 with a mean of  $0.37 \pm 0.12$ ). Although all 9 females in Subgroup LP continued to consume at least some of their own placenta during parturition, only 1 of the females (an eater during parturition) ate donor term placenta offered just after parturition. In summary, females in the pregnant, LiCl-treated group continued to consume about 1/3 of their own placentas during parturition and were not likely to consume donor term placenta presented after parturition.

An important determinant of the effectiveness of TAC in Subgroup LP was the success of the experimenter in preventing placentophagia during the first parturition, prior to instituting TAC: the Spearman rank order correlation coefficient between the number of placentas eaten during the first parturition and the placentophagia index during the second was +.94 ( $p < 0.01$ ).

All 6 females in Group SP showed complete consumption of their own placenta during the second parturition. By contrast, 5/9 of the LiCl group (Group LP) expressed an aversion to their own placenta during parturition ( $p = 0.04$ , Fisher test). Five of the 6 females in the saline group consumed the donor term placenta presented just after parturition. The difference between this group and the LiCl group on the postpartum test (1/9) was significant ( $p = 0.01$ ; Fisher test). In summary, the administration of TAC during the first parturition (particularly if placentophagia only occurred in conjunction with LiCl) altered placentophagia both during and just after the second parturition.

#### *Observation of Maternal Behavior*

During the perinatal period, the 4 females in the LiCl-injected group (Subgroup LP) that had exhibited placentophagia during parturition also severed the umbilical cords and cleaned all their pups. Among the 5 animals showing an aversion to placenta, 2 failed to clean 1 pup each and 4 failed to sever the umbilical cords on a large number of pups in their litters. Although all the females in the saline-injected group (Group SP) exhibited placentophagia during the perinatal period, 2 of the 6 females did not clean all the pups in their litters. With the obvious exception of placentophagia, no significant difference in perinatal maternal behavior could be detected between the saline and LiCl-injected groups.

When observed at 2 days postpartum, all the females in the LiCl-treated group (Subgroup LP) built nests, nursed their young and retrieved their litters within the 15 min test. Five females lost at least 1 pup from their litters. The number of pups lost tended to be small, ranging from 1 to 3, with the mean being  $2.0 \pm 0.4$ . Although most of the females in the saline-injected group (Group SP) continued to show maternal behavior at 2 days postpartum, there was a high incidence of pup mortality. All the animals in this group lost at least 1 pup from their litters. The number of pups lost tended to be large, ranging from 1 to 16, the mean being  $5.7 \pm 2.2$ . Although a larger number of females in the saline groups exhibited pup loss when compared to the LiCl-treated group, this difference was not significant ( $p = 0.09$ ; Fisher test).

#### *Two-week postpartum test*

All the females in the LiCl-treated group (Subgroup LP) exhibited placentophagia in the test 52 days after TAC. Since only 4 of the 9 females had eaten placenta during parturition, this increase was significant ( $p = 0.03$ ; McNemar test). All 6 females in the saline group (Group SP) exhibited placentophagia in the test after the control interval. The saline-injected females showed no change from their response to placenta at parturition.

#### *Response of nonpregnant females*

Females in Subgroup LP had been given an injection of LiCl following the consumption of donor term placenta

after the first parturition. During the test after the first control interval, which occurred at 38 days after the first parturition, all of the LP females continued to exhibit placentophagia.

A comparison between Subgroup LP and Subgroup LP revealed a significant difference ( $p = 0.02$ , Fisher test). Whereas more than half of the females in the pregnant group manifested an aversion to placenta at the second parturition, none of the females in the nonpregnant group gave any indication of an aversion after the first control interval.

The response of the nonpregnant group in Experiment 3 (LP) can be contrasted with the response of the nonpregnant groups in Experiment 1 (Subgroup CP) and in Experiment 2 (Group P). Whereas the nonpregnant groups from Experiments 1 and 2 exhibited an aversion after the first control interval, the nonpregnant group in Experiment 3 did not. The comparison between LP and CP was significant ( $p = 0.004$ ; Fisher test) as was the comparison between LP and P ( $p = 0.009$ ; Fisher test).

When observed after the second control interval (52 days after conditioning) all females in Subgroup LP continued to exhibit placentophagia as in the test after the first control interval. The results of the second control interval (2 week postpartum for P females) tests performed on the 3 groups in Experiment 3 revealed that all groups were exhibiting placentophagia at this time.

To summarize the results of Experiment 3, a taste aversion to placenta, induced at parturition, was expressed at the next parturition; the expression of the aversion was contingent upon limiting the number of placentas eaten during the first parturition (just prior to TAC), females eating more than one placenta during the first parturition showing no aversion to placenta during the second. An aversion conditioned at parturition was not expressed during a subsequent nonpregnant period. The saline-injection procedure did not lead to the expression of an aversion to placenta during the second parturition.

## DISCUSSION

The role of prior parturitional experience with placenta can be elucidated by making comparisons between inexperienced (Exp. 1) and experienced (Exp. 2) females. Nonpregnant females, even those with prior parturitional experience with placenta, can acquire an aversion to placenta by TAC. The aversion was induced with equal facility in both virgins and nonpregnant primiparae. The aversion could still be observed in both groups after an interval of 25–30 days. Thus nonpregnant females can acquire an aversion to placenta, and express it on both a short-term and long-term basis.

If females were impregnated after the completion of TAC, the aversion to donor placenta could alter the consumption of naturally-delivered placenta during a subsequent parturition. TAC was particularly effective in modifying parturitional placentophagia when TAC was induced in virgins and tested at the first parturition. These females had very little exposure to safe placenta before the onset of conditioning, learned safety of placenta having been kept to a minimum. After conditioning, these inexperienced females clearly responded to donor placenta and their own placenta in the same manner, refusing to consume either variety. Females that lacked parturitional experience with placenta did not demonstrate an ability to distinguish

between their own and the donor placenta: the aversion conditioned to donor placenta (surgically removed) generalized to their own placenta (normally delivered).

Experienced females, on the other hand, had consumed placenta during their first parturition, which had occurred before the induction of the aversion; primiparae had the opportunity to develop learned safety to placenta prior to TAC. Although an aversion to donor placenta was acquired by nonpregnant primiparae, they did not express the aversion to their own normally-delivered placenta at the subsequent parturition. They did express an aversion, however, to donor placenta presented immediately after parturition. The primary effect, therefore, of prior parturitional experience seems to have been to enable the primiparae to distinguish between their own and donor placenta (or perhaps between normally-delivered and surgically-removed placenta) as evidenced by the ease of inducing TAC and by the difference in response between the parturitional observation and the immediately-postpartum test.

In Experiment 3, TAC occurred immediately after delivery in primiparae, rather than in a nonpregnant state. The potential for expression of the aversion during a second parturition may have been enhanced by conditioning and testing the aversion under the same conditions (i.e., parturition). The results of Experiment 3 indicate that if,

during the first parturition, unpaired ingestion of placenta prior to TAC is prevented, then a single pairing of placenta with LiCl-induced illness can reduce the incidence of placentophagia during the second parturition.

The aversion induced to normally-delivered placenta, in Experiment 3, was expressed when fresh normally-delivered placenta was available, such as at delivery and during the immediately-postpartum test (the stimulus for this test was normally-delivered placenta only in Experiment 3), but not when frozen and thawed normally-delivered placenta was available in the test that occurred 44 days after TAC. The response at 44 days may have been due to a recovery from the aversion, may have reflected a discrimination between fresh and frozen term placenta, or may have been due to the fact that the 44 day test did not occur when the female was parturient. The present design cannot discriminate among these alternatives.

As determined by observations of concomitant maternal behavior, conditioned aversion to placenta appeared not to cause severe disruptions in the sequencing of maternal behavior. The aversion appeared to modify only the ingestion of placenta, leaving pup-licking, removal of the umbilicus and amnion, retrieval, and nest-building, intact. The partial or complete absence of placentophagia during parturition did not appear to prevent the female from providing adequate, though broadly defined, maternal care.

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