Effects of Hypothalamic Knife Cuts and Experience on Maternal Behavior in the Rat

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FRANZ, J. R., R. J. LEO, M. A. STEUER AND M. B. KRISTAL. Effects of hypothalamic knife cuts and experience on maternal behavior in the rat. PHYSIOL. BEHAV. 35(5):529-640, 1986.—Recent investigations suggest that the disruption of placenotaphagia, pup-directed maternal behavior, and nestbuilding seen after lesions of the medial preoptic area (MPO) or the lateral hypothalamus may be due to the interruption at different points of a single longitudinal neural system mediating these behaviors. To test this, we compared the effects of knife cuts on the lateral border of the MPO, and of the posterior medial forebrain bundle (MFB), with asymmetrical cuts combining a unilateral MPO cut with a contralateral MFB cut. We observed placenotaphagia, nestbuilding, and pup-directed maternal behaviors at and after parturition in both primiparous and biparous rats. In primiparous MPO cut (a) disrupted placenotaphagia, (b) delayed the onset of grooming and puplicking, and (c) eliminated nestbuilding. MFB cuts (d) disrupted placenotaphagia, (b) delayed the onset of maternal behavior, and (c) eliminated nestbuilding. Asymmetrical cuts (e) disrupted placenotaphagia, and (b) delayed the onset of maternal behavior. In biparous rats, MPO cuts eliminated nestbuilding and reproductive MFB cuts (d) disrupted placenotaphagia, and (b) eliminated nestbuilding. Asymmetrical cuts (c) delayed nestbuilding. These results suggest the involvement of a longitudinal neural system in the production of immediate pup-directed maternal behavior, placenotaphagia, and nestbuilding in parturient primiparous, but not in the immediate onset of placenotaphagia and maternal behavior in maternally experienced rats.

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THE medial preoptic area (MPO), which lies immediately rostral to the anterior hypothalamus, is the brain region that is most strongly implicated in the control of maternal behavior (see [40] for review). Although a unified theory of the role of the MPO in maternal behavior has not yet emerged, its involvement has been demonstrated in numerous studies. The MPO appears to be important in the gradual induction of maternal behavior produced by excessive exposure to stimuli emanating from rat pups, such as that produced in virgin rats by housing them with pups. It also appears to be the locus at which steroid hormones act to intensify the effect of extrinsic stimuli on the pup-directed maternal behavior almost immediately upon exposure to pups, such as that evident at delivery [15, 17, 19, 20, 24, 38, 39, 41, 44, 61].

The medial preoptic area, more specifically the fibers coursing through it, may also be implicated in the control of maternal behavior. Paraventricular knife cuts placed between the medial and lateral preoptic areas produce disruptions in maternal behavior that are similar to those produced by MPO lesions; the lateral connections of the MPO appear to be critical for maternal behavior produced by pup exposure alone, as well as that facilitated by hormonal manipulations [29-31, 39, 41, 42, 54, 57, 60].

Although many of the neuroanatomical sites from which the MPO receives lateral afferent projections, and to which the MPO sends lateral efferent projections, have been described, the specific pathways critical for maternal behavior have not yet been delineated. Studies on efferent projections have shown that fibers travel laterally out of the MPO through the lateral preoptic area, and substantia innominata to the medial amygdala, laterally through the supraoptic commissure of Meynert (located just above the optic tract) into the amygdala, or dorsolaterally through the stria terminalis to the medial amygdala. Descending axons travel laterally out of the MPO through the medial portion of the medial forebrain bundle (MFB), through which they descend to terminate in the median eminence, arcuate nucleus, medial hypothalamus (paraventricular, dorsomedial, and ventromedial nuclei), lateral hypothalamus, ventral premammillary, and supramammillary nuclei, central gray of the

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mesencephalon, ventral tegmental area, median and dorsal raphe nuclei, midbrain reticulata formation, and pontine central gray (5, 10, 11, 38, 56, 59).

Since many of the descending projections of the MPO course through the MFB, and since the MFB is an important pathway connecting the basal forebrain and hypothalamus with lower brainstem areas (see [35, 36] for review), it is not surprising that bilateral knife cuts that sever the connections between the MPO and the MFB disrupt maternal behavior [29-31, 39, 41, 57, 66]. Numan and his colleagues have recently suggested that a particular subset of these descending lateral MPO projections—those that course laterally into the lateral preoptic area, then from there through the dorsal MFB to the ventral tegmental area—are critical for postpartum maternal behavior [42, 43, 45]. Numan et al. [42] concluded that although surgical interruption of MPO efferents that descend directly to the brainstem via the ventral portion of the MFB may induce some transient effects on postpartum maternal behavior, these efferents are not essential for such maternal behavior. On the other hand, since surgical damage of the dorsal portion of the MFB, perhaps because of the interruption of MPO efferents within the MPO-lateral preoptic-lateral tegmental circuit, produces severe deficits in maternal behavior, this pathway is critical for the expression of postpartum maternal behavior.

There is also some evidence to suggest that other lateral MPO projections, which do not descend through the MFB but instead descend in the region of the topographic commissures, may also be important for the onset of pup retrieval and nursing behavior in both postpartum and virgin rats continuously housed with foster pups [20-31].

Since the initiation of placentophagy (ingestion of the afterbirth) and infant-directed caretaking behavior occur in close proximity or after parturition in most mammals, and since placentophagy can be conceptualized as both a maternal and an ingestive behavior, one might expect that these behaviors are governed by related physiological mechanisms. Furthermore, research has confirmed that both feeding and drinking behaviors for placentophagy in primiparous are different from those in multiparous, and control mechanisms in virgin rats are different from those in parturient rats (see [22] for review). Kristal and his colleagues found that placental lesions that eliminated feeding and drinking behaviors for placentophagy in nonpregnant nulliparous pregnant with placenta obtained from donor females, and in rats giving birth for the first time, but did not eliminate placenta in nonpregnant multiparous presented with donor placenta, or in rats giving birth for the second time [21, 37]. Likewise, Numan and Kristal [38] found that median MPO lesions had no effect on placentophagy in virgin rats, but that such lesions did disrupt placenta in placenta at parturition. They also found that such MPO lesions also produce deficits in postpartum maternal behavior. Taken together, the results of these studies suggest that the physiological mechanisms that regulate placentophagy in nonpregnant rats are different from those that regulate placentophagy in parturient rats, and that postpartum maternal behavior and placenta may have some common neurochemical aspects.

Since MPO lesions disrupt parturitional placenta as well as postpartum caretaking behavior in primiparous, and since lateral hypothalamic lesions eliminate parturitional placenta in primiparous, it is plausible that connections between the MPO and the lateral hypothalamus are important for the onset of both behaviors at parturition. With reduced
either eat donor placenta when first exposed to it, or they avoid it (for review, see [22])—knuckle cuts were made in rats that were spontaneously attracted to placenta as virgins (placentophages), as well as in those that avoided it (nonplacentophages).

Experiment 2 was conducted to determine the influence of parturitional experience on the behavioral effects observed in Experiment 1.

EXPERIMENT 1

METHOD

Subjects

Sixty breeder rats (n = 45), pup-donors, and placenta-donors were Long-Evans females, 3–6 months old, born and raised in our laboratory. Their mothers had been purchased from the Charles River Breeding Laboratories. Each rat was housed individually in a 24 × 19 × 18 cm wire-mesh cage with food (Charles River Rat/Mouse Hamster Formula 3000) and water available ad lib except as indicated below. All rats were maintained on a 14 hr on/10 hr off light cycle with the on phase beginning at 0600 hr (EST).

Procedure

Placentophagia test. Daily vaginal smears were obtained from each rat, beginning at about 60 days of age. After verification of normal estrous cyclicity, the rat was given a placenta in a test cage. The placenta was observed surgically from CO₂-killed, Day 21 pregnant females, then placed in a small plastic vial with a few drops of physiological saline and frozen at −20°C until used. A few minutes before presentation, the placentas were thawed and warmed to about 30°C then placed individually into glass dishes and presented for 15 min to the rats, which had been placed in wire-mesh cages in a quiet room 2 hr earlier [23]. After the subject’s response to placenta was recorded, it was returned to its home cage. The testing procedure was terminated when the rat ate placenta and was classified as a placenta- phage, or after refusal to eat placenta in three consecutive daily tests, in which case she was classified as a nonplacentophage. Previous research has indicated that once a virgin eats placenta, she is likely to eat it whenever it is made available subsequently; virgins that refuse to eat on all three test days are not likely to eat placenta on subsequent occasions except during delivery or unless coerced by food deprivation [23].

Maternal-behavior test. One to two weeks after the placenta test, each rat was habituated overnight to a 45 × 19 × 25 cm plastic cage containing 3 cm of coarse saw- dust and fitted with a standard wire grill-top containing food and water. The next day, four foster pups, 3–8 days of age, were scattered about the floor of the cage, and the rat’s responses during the next 15 min were noted. A rat was excluded from the experiment if she was observed, attacked, or ate any of the pups. Spontaneous piercings and cannibals constitute a very small proportion of our rat colony; because such animals are identified in numbers too low to be distributed evenly across groups, they were instead excluded from the experiment. At the end of this test the pups were returned to their mother and the test rat was returned to its home cage.

Confirmation of nonplacentophagia status. Each rat in

cluded as a nonplacentophagia in this experiment was asked to demonstrate a refusal to eat placenta in a fourth test conducted as described in the placenta test. This 1-day test occurred on a non-proestrus day of the estrous cycle 1 to 2 weeks prior to time-breading. Knuckle-cut surgery. Bilateral knuckle cuts were made stereotaxically in the anesthetized with methoxyflurane (Metofane, Pitman-Moore) on the afternoon of Day 20 of pregnancy. The knife was a modified version of that described by Schiøtt and Graumann [3] and consisted of a 28-ga gauge cannula bent to have a fine tip, the ends of which were so fashioned that a 38-ga stainless steel wire forced through the cannula extended in the direction of the curved end. For MFO cuts the cannula was inserted at 2.2 mm anterior to bregma and 1.3 mm lateral to midline, and lowered until the bottom of the wire would, when extended, reach 7.6 mm below the skull surface [47]. The wire was then forced through the cannula and out the curved end in the sagittal plane to a length of approximately 1.2 mm from the cannula tip. The cut was made by lowering the cannula with the extended wire a distance of 2.0 mm. The cannula was then raised to 7.0 mm below the skull surface, the wire was retracted, and the cannula was withdrawn from the brain. This procedure was then repeated on the other side of the brain. MFB cuts were made by inserting the cannula 1.2 mm posterior to bregma and 2.2 mm lateral to midline, lowering it to 8.6 mm below the skull surface, extending the inner wire approximately 1.5 mm toward midline, lowering the knuckle assembly to 9.0 mm below the skull surface, raising it to the initial point of expansion, retracting the wire, and withdrawing the cannula from the brain. The procedure was then repeated on the other side of the brain. Control rats receiving MFO or MFB sham cuts were subjected to the same surgical treatments described above, except that the inner wire was not extended. Each rat in the ASYM Group received a unilateral MFB cut and a contralateral sham cut, and each rat in the ASYM-Control Group received a unilateral MFB cut and a sham MFB cut, and all other half received a unilateral MFB cut and a sham MPO cut.

Measurements of postoperative food and water intake. After the surgery, each rat was weighed and placed in a wire-mesh cage containing measured amounts of food and water. Twenty-four hours later, on the 21st day, the weights and food and water intakes were determined. Debilitated rats were excluded from further experimentation. Healthy subjects were moved to plastic cages containing a small amount of sawdust, a supply of paper strips, and food and water. The cages were placed in a room separate from the main colony that was equipped with a remotely controlled low-light video camera.

Placentophagia, maternal-behavior, and restructuring observations and testing. Beginning on Day 22 of pregnancy each rat was observed for parturition during frequent check-ins. At delivery, continuous observations were made to determine the incidence of placenta-phagia, the incidence of cannibalism, and the condition and number of pups. The time of onset and duration of parturition were also noted. One hour after completion of delivery, each mother was presented for 15 min with 5 donor placenta in a glass dish.

Six hours after completion of delivery, each mother was weighed, the number of delivered placentas that remained un eaten was noted, the litter and remaining placentas were removed, and the average pup weight was determined. Then 4 foster pups, 3–8 days old, were scattered about the cage, and the during the next 15 min retrieval, pup licking, and crouch-
ing over pups were noted. Afterward, any exiling nest was destroyed, and 1 hr later the presence or absence of a rebuilt nest was noted.

On each of the next five days, at 0900 hr each mother was weighed and her pups removed and replaced with a group of four freshly nourished pups. Maternal behavior and nest-building tests were conducted twice daily, between 0900 and 1000 hr and between 2100 and 2200 hr. A total of 11 tests was conducted: the first test on which nestbuilding or any component of maternal behavior was observed was taken to be the latency for the onset of that behavior. After Test 11 on postpartum Day 3, each mother was separated from her pups and placed into a wire-mesh cage with food and water.

Test for hyperphagia. To determine whether hyperphagia was produced by the knife cuts, measurements of food intake, water intake, and body weight were taken daily for 10 days beginning on the third day after the rats were transferred to wire-mesh cages. These measurements were initiated at this time, rather than immediately after surgery, in order to avoid the confounding effects on food intake of the increased energy requirements associated with gestation and lactation [14]. The 3-day interval between weaning of pups and initiation of testing was chosen on the basis of Fleming's finding [40] that the elevated level of food intake characteristic of lactating females does not decline to prepartum levels until 24–48 hr after weaning.

During the test period, the rats were maintained on tap water and a high-fat diet, available ad lib, consisting of two parts ground laboratory chow and one part vegetable shortenings (Crisco). This diet is highly palatable to rats, and therefore provides a sensitive measure of hyperphagia [12]. The food was presented in baby food jars attached to the front of each cage. Food jars and water bottles were weighed daily, and food and water intakes were determined by calculating difference weights over each 24-hr period. Daily food intake was corrected for spillage. The day on which a rat was first observed to eat was designated Day 0 of hyperphagia testing. Intake and body weight measures were continued through Day 10.

Histological analysis. At the conclusion of the test for hyperphagia, rats with knife cuts were anesthetized with an overdose of pentobarbital and perfused intracardially with physiological saline followed by 10% formalin. The brains were then removed, fixed in formalin, cut at 40 μm on a cryostat, and every fifth section stained with cresyl violet or formal-thionin. The location and extent of the knife cuts were then verified microscopically.

RESULTS

For statistical analyses, data from the ASYM-Control Group were pooled with data from the MPO and MBF sham groups, since these groups were not different from one another.

The analysis of the effect of pre-breeding response to placenta was performed before ASYM preparations were run. The analysis was a 2 × 3 design (Placenta Preposition [placenta/no placenta] × Knife Cut [MPO, MBF, SHAM]). Where we determined that there were no significant main or interaction effects of placenta preposition on placenta or maternal behavior, data from four groups were pooled for all subsequent analyses. Because placenta preposition had no effect, and because placenta treatments make up the large majority of our colony, all the rats receiving ASYM cords were placenothalyses.

Postoperative Food and Water Intake

Nine rats (3/15 with MPO cuts, 5/15 with MBF cuts, and 1/15 with SHAM cuts) were dropped from the experiment on Day 21 of pregnancy due to extreme debilitation, which included aphagia and adipsia. Subsequent histological examination revealed that debilitation was probably caused by incorrectly placed knife cuts, many of which produced hemorrhage and/or widespread hypothalamic damage. For each of the remaining rats, 24-hr postoperative food and water intakes were calculated as percentages of Day 20 body weight for groups of primiparas rats with MPO, MBF, ASYM, and SHAM cuts. An analysis of variance indicated a significant effect of surgery on food intake, F(3,49) = 3.67, p < 0.05, with food intakes (mean percentage of body weight) of 4.8 ± 0.78%; 7.7 ± 0.98%; 8.3 ± 0.78%; and 5.9 ± 0.9%, for MPO, MBF, ASYM, and SHAM Groups, respectively. Subsequent pairwise comparisons (all pairwise comparisons after ANOVAs were done by a Newman-Keuls test, p < 0.05) revealed that rats with MPO cuts ate significantly less than those with MPO or ASYM cuts, but not significantly less than the SHAM Group. The MBF and ASYM Groups did not eat significantly more than the SHAM Group. There were no significant differences in water intake among the four groups, F(3,49) = 1.6, p > 0.05. Mean water intake (percentage of body weight) ranged from 13.0 ± 1.0% for the SHAM Group to 17.7 ± 2.76 for the ASYM Group.

Timing of Parturition and Condition of Offspring

There were no statistically significant differences among the four surgical groups with respect to the duration of parturition, F(3,49) = 1.45, p > 0.05. Mean durations ranged from 74.4 ± 8.3 min for the MPO Group to 107.9 ± 23.3 min for the ASYM Group. The data on gestation length were compiled as percentages of each group that delivered in each hour on Day 22, Day 23, or Day 24 of pregnancy. ANOVA revealed that there were no statistically significant differences among the four surgical groups, F(3,49) = 1.6. Based on data gathered across all 4 groups, 15.8% of the rats delivered on Day 22, 64.1% delivered on Day 23 and 20.5% delivered on Day 24.

There were no significant differences among the four surgical groups with respect to litter size, F(3,49) = 0.0 (means ranged from 8.9 ± 1.1 pups for the ASYM Group to 9.9 ± 1.9 for the SHAM Group); percentage of each litter born alive, F(3,49) = 2.2, p > 0.05 (means ranged from 54.9 ± 11.7% for the MPO Group to 64.3 ± 4.8% for the SHAM Group); or live pup weight, F(3,49) = 2.18, p > 0.05 (means ranged from 4.2 ± 0.6 g for the MPO Group to 6.0 ± 0.2 g for the ASYM Group). Cannibalism was extremely rare. The three rats that did cannibalize (a placenothalypse with MPO cuts, a placenothalypse with MBF cuts, and a nonplacenothalypse with MBF cuts) are only dead pups and never attacked live pups.

Placenothalypse

Delivered placenta. The percentage of groups of nonplacenothalyses with MPO, MBF, and SHAM cuts that ate all their delivered placentas were not significantly different from those of placentas with comparable cuts, F(1,33) = 1.0. However, there were significant differences
among the four surgical groups, F(3,49)=7.25, p<0.01. Ninety percent of the rats in the SHAM Group ate all their
placentas, but only 33% of each of the knife-cut groups ate all their placentas. Pairwise comparisons indicated that there
were no significant differences among the MPO, MFB, and ASYM Groups, but each of the three groups was significantly
different from the SHAM Group.

Donor placentas. The percentages of groups of nonplacentophages with MPO, MFB, and SHAM cuts that
ate donor placenta 1 hr after parturition were not signifi-
cantly different from those of placentalophages with com-
parable cuts, F(1,33)=1.0. When data from placentalophages
and nonplacentophages were combined, there were no signif-
ificant differences among the four surgical groups,
F(3,49)=1.0. The percentage of each group that ate donor
placenta was: MPO: 58%; MFB: 67%; ASYM: 88%; SHAM: 59%.

Pup-Directed Maternal Behavior and Nestbuilding

At the end of each of the 11 maternal behavior and
nestbuilding tests, each rat was scored as having begun (1) or
not having begun (0) to display (a) retrieving of all four pups,
(b) crouching over one or more pups, (c) pup-licking, and (d)
establishing. For each of these dependent measures, the
resulting set of dichotomous data was analyzed using a
1-within, 1-between ANOVA [35] with Locus of Knife Cut as
the between factor and Test as the within factor.

Retrieving. Figure 1 shows the cumulative percentage of
rats in each group that retrieved all four foster pups. There
were significant differences among the groups on Test 1,
F(3,11)=17.72, p<0.01, in that the percentages of the MPO,
MFB, and ASYM Groups that retrieved during this test were
significantly lower than that of the SHAM Group, but were
not different from each other. Over the course of testing,
there was a significant increase in the percentage of rats
retrieving in the MPO Group, F(1,49)=15.48, p<0.01, and
in the ASYM Group, F(1,49)=7.18, p<0.01. That is, rats
with MFB or ASYM cuts showed a delayed onset of retriev-
ing; at least half of each of these groups had retrieved pups
by Test 11 on Day 3 postpartum.

Crouching. Figure 2 shows the cumulative percentage of
rats in each group that adopted a crouching posture over one
or more pups. There were significant differences among the
groups on Test 1, F(3,10)=11.39, p<0.01. The percentages
of rats in the MPO, MFB, and ASYM Groups that crouched
on this test were significantly lower than that of the SHAM
Group but were not different from each other. However,
over the course of testing, there were significant increases in
the percentage of rats crouching over pups for each of the	hree knife-cut groups, MPO: F(10,49)=10.79, p<0.01;
MFB: F(10,49)=8.13, p<0.01; ASYM: F(10,49)=9.18,
p<0.01. By Test 11, there were no significant differences
among the four groups, F(3,10)=2.19, p>0.05.

Pup-licking. Figure 1 shows the cumulative percentage of
rats in each group that licked pups. The effects of cuts
on pup-licking were similar to the effects on crouching. There
were significant differences among the groups on Test 1,
F(3,11)=13.33, p<0.01. The percentages of rats in the three
knife-cut groups that licked pups during this test were signifi-
cantly lower than that of the SHAM Group but were not
different from each other. Over the course of testing, how-
ever, there were significant increases in the percentage of
rats licking pups in each of the three knife-cut groups, MPO:
F(10,49)=15.02, p<0.01; MFB: F(10,49)=14.48, p<0.01;

FIG. 1. Cumulative percentages of groups of primiparae rats, after
knife-cut surgery, that displayed retrieving, crouching, pup-licking
and nestbuilding across tests.

ASYM: F(10,49)=8.04, p<0.05. By Test 11, there were no
significant differences among the groups, F(3,11)=1.0. Nestbuilding. Figure 1 shows the cumulative percentage of
rats in each group that built nests. There were significant
differences among the groups on Test 1, F(3,11)=11.81,
p<0.01. The percentages of rats in the MPO, MFB, and
ASYM Groups that built nests on this test were significantly
lower than that of the SHAM Group but were not different
from each other. The percentage of rats that built nests did not
increase over testing for the MPO Group, F(10,49)=1.13,
p>0.05, nor for the MFB Group, F(10,49)=1.70, p>0.05,
but did increase for the ASYM Group, F(10,49)=10.70,
p<0.01. MPO or MFB cuts elimi-
nated nestbuilding for the entire 11-day test period, but
ASYM cuts only delayed the onset of nestbuilding; half of
this group had built nests by Test 11 on postpartum Day 5.

Test for Hyperphagia. Rats with MFB cuts were hyper-
phagic on all 10 days of exposure to the high-fat diet.
They ate an average of 9.5% of their body weight on each
day of testing, compared with 6.0%, 6.5% and 5.5% for
rats with MPO, ASYM, and SHAM cuts, respectively,
F(3,49)=34.92, p<0.01. For each rat, body weight on Days
1–10 was calculated as percentage of body weight on Day 0.
Significant differences among the groups were apparent as
early as Day 2, F(3,77)=3.56, p<0.05. By Day 10, the MFB
rats weighed almost 140% of the Day 0 baseline, whereas
the other groups averaged 109% of baseline.

Histological Analysis

Examination of brain sections revealed that the MPO cuts
were similar to those described by Numan [39]. There was
little variability in the extent and location of MPO cuts. They
were bilaterally symmetrically located between 1.3 and 1.5
mm lateral to midline, just medial to the supraoptic nuclei
[47]. In 10 of the 12 rats, the cuts began at the rostral end
of the MPO (A 7.8–8.2) and extended caudally into the anterior
FIG. 2. Schematic representation of a typical MPO knife cut.

FIG. 3. Schematic representation of a typical MF5 knife cut.
hypothalamic area (A 6.8-7.0) containing most of the mediolateral connections of the MnP between the anterior commissure and the optic chiasm (see Fig. 2). In the other two rats, the cuts began in the middle of the anterior-posterior portion of the MnP (A 7.4-7.6) and extended well into the anterior hypothalamic area (A 6.6-9.8).

MFb cuts were located just posterior to the posterior tips of the ventromedial nuclei in a coronal plane that varied from A 4.5 to 3.0 (47). The MFB was cut bilaterally in all 12 rats from L 0.7 to L 1.7 and from just ventral to the hippocampal-amygdaloid tract (H - 3.0) to the base of the brain. Thus, the fornix and parafornical MFB were severed bilaterally in all 12 rats with MFB cuts (see Fig. 2). In four rats the cuts extended bilaterally into the medial hypothalamus almost to the third ventricle (L 0.3); in two of these rats the cuts extended bilaterally as far lateral as L 1.2-2.0.

Four of the rats in the ASYM Group had unilateral MPO cuts that were similar to those described above for rats with bilateral MPO cuts. The other four rats in this group had slightly longer cuts, which extended caudally to A 6.4. The location and extent of unilateral MFB cuts in rats in the ASYM Group were the same as described above for rats with bilateral MFB cuts.

The rats with MPO cuts that cannalized some of their young was the only one with cuts that extended rostrally into the diagonal band of Broca (A 8.2). This rat was also among the four with MPO cuts that ate all their delivered placenta. However, comparison of the knife-cut damage in these rats with that in rats that did not eat their delivered placenta revealed no relationship between knife-cut placement and parturitional placenaphagia. Similarly, no relationship was found between placement of MPO cuts and the incidence of nebstalking (two rats with MPO cuts had built nests by Test 11).

**DISCUSSION**

All three knife cuts reduced the likelihood that a rat would eat all of her delivered placenta, and prevented the display of retrieving, pop-licking, and nebstalking for the postpartum. These findings suggest that connections between the MnP and the posterior MFB constitute a functional longitudinal neural system that is involved in producing the rapid onset of both the ingestive and caretaking components of parturitional behavior prior to delivery in primiparous rats. That the knife-cut-induced deficits in the onset of placenaphagia, maternal behavior, and nebstalking were caused directly by disruption of a neural system specifically involved in producing the rapid onset of these behaviors is supported by the finding of no differences between the SHAM Group and the three knife-cut groups with respect to postoperative food and water intake, timing of parturition, or condition of offspring.

It might be that the observed deficits in placenaphagia, retrieving, pop-licking, and nebstalking were merely an indirect result of a general disruption of oral behaviors. However, this seems unlikely, since cuts disrupted crouching, a non-oral caretaking behavior, but did not disrupt feeding and drinking, oral non-caretaking behaviors.

The conclusion that the onset of parturitional behaviors is controlled by a longitudinal neural system was based on the criterion that cuts at all three placements must produce the same behavioral deficits. This criterion was also applied in determining whether deficits observed during the subsequent 5-day test period could be attributed to the disruption of a longitudinal system.

By the end of testing on Day 5 postpartum, many of the rats with asymmetrical cuts had begun to display full maternal responsiveness, including retrieving, crouching, pop-licking, and nebstalking. This suggests that the eventual display of maternal behavior and nebstalking does not depend on the longitudinal system found to be necessary for the rapid onset of these behaviors at parturition. The eventual display of maternal responsiveness in rats with asymmetrical cuts, and the eventual display of some but not all maternal behaviors by the other knife cut groups (e.g., pop-licking but not nebstalking) are thought to be similar to the eventual display of maternal responsiveness in virgin rats in response to pup stimuli. Although it is possible that within-group differences in latency to respond to pups could be explained by differential rates of recovery from surgical trauma, other behavioral measures indicate satisfactory recovery from surgery, and the observed latency differences are also consistent with the variabilty ic rates of maternal sensitization typically observed in virgin rats.

In contrast to the temporary effects of ASYM cuts, both MPO cuts and MFB cuts produced behavioral deficits that appeared to be permanent. Consistent with the findings of previous investigations (39,57), MPO cuts eliminated retrieving and nebstalking for the entire 5-day test period. Since these effects were not duplicated by ASYM cuts, they cannot be attributed to the disruption of a longitudinal system. That is, the laterl connections of the MPO that are important for retrieving and nebstalking are apparently not those which pass through the posterior MFB. There is, in fact, evidence suggesting that MPO projections that descend in the region of the supraoptic commissures, and that were spared in rats with ASYM cuts, are critical for the display of retrieving and nebstalking (29-31). Despite these long-term deficits in retrieving and nebstalking, however, most of the rats with MPO cuts eventually began to lick and crouch over pups. These results provide support for the idea that different components of maternal behavior are controlled by separate neural systems (57).

MFB cuts were associated with nebstalking for the entire 5-day test period and produced hyperphagia on the high-fat diet. Neither of these effects was duplicated by ASYM cuts, and therefore cannot be attributed to the disruption of a longitudinal system. The production of hyperphagia by MFB cuts is not surprising, since MFB projections extend into the hypothalamic area produce hyperphagia (29,52). Although the production of hyperphagia by MFB cuts causes some doubt on the specificity of the deficits in parturitional behavior exhibited by rats with these cuts, this problem of interpretation does not have any serious implications for the conclusion that a longitudinal system mediates the onset of parturitional behaviors; rats with ASYM cuts exhibited deficits in these behaviors but were not hyperphagia.

On the basis of observations made at 6 hr postpartum that rats with knife cuts had not eaten all their delivered placenta, were not maternally responsive toward foster pups, and did not build nests, we have concluded that a longitudinal system participates in the control of both the ingestive and caretaking components of parturitional behavior.

In some respects, parturitional behavior of rats with knife cuts was similar to that of SHAM controls. During delivery of individual pups, they adopted the head-between-the-heels position and pulled as pups 2nd placenta as they were delivered, licking them as they emerged. They some-
times ate the placentas. However, rats with cuts did not eat their placentas or lick their pups with the celerity and thoroughness characteristic of control rats. For example, between deliveries of individual pups, rats with cuts ignored both pups and placentas much of the time. On the occasions that they did attend to pups, they sniffed and licked them, but did not engage in the vigorous grooming of pups seen in control rats. Nor did rats with cuts build nests or gather the pups together, even after parturition had been completed. Instead they typically went to sleep, leaving pups scattered about the cage, some still covered with membranes and some with placenta still attached.

Finally, comparison of the effects of knife cuts on placentophagia in females that were either placentophages or nonplacentophages or virgin revealed that prior ingestion of placentas, in the absence of parturitional experience, does not prevent the disruption of parturitional placentophagia by MPO or MFB knife cuts.

**EXPERIMENT 2**

A number of lines of evidence have suggested that the experience of delivery and rearing the first litter protects placentophagia and maternal behavior from disruption produced by manipulation of physiological mechanisms shown to be necessary for their occurrence during the first parturition. In order to determine whether prior parturitional experience influences the effects of hypotalamic knife cuts on parturitional behavior, this experiment was designed to investigate the effects of MPO, MFB, ASYM, and SHAM cuts in biparous rats.

**METHOD**

**Subjects**

Experimental rats (n=36), pup-donors, and placenta-donors were Long-Evans females, 3-6 months old, born and raised in our laboratory ( offspring of pups purchased from Charles River Breeding Laboratories) and housed and maintained as described in Experiment 1.

**Procedure**

At the onset of the experiment, each experimental rat had been determined, by the procedure described in Experiment 1, to be a placentophagous as a nonpregnant nulliparas, and had subsequently given birth to a litter and had raised it to weaning. On two weeks after having her pups weaned at 25 days of age, each experimental rat was bred, subjected to knife-cut surgery, and tested according to the procedures described in Experiment 1.

**RESULTS**

**Postoperative Food and Water Intake**

Four of 12 rats with MFB cuts were dropped from the experiment on Day 21 of pregnancy due to extreme debilitation. The mean food intake as percentage of body weight for groups of biparous rats with MPO, MFB, ASYM, and SHAM cuts were 5.2±0.88, 6±0.78, 5.7±0.8%, 5.6±0.3%, respectively. The mean water intakes (percentage of body weight) for these groups were: MPO: 18.0±3.7%; MFB: 12.8±2.0%; ASYM: 14.4±1.9%; SHAM: 14.1±1.2%. There were no significant differences among the groups in food intake, F(3,32)<1.0, or water intake, F(3,32)<1.0.

**Tuning of Parturition and Condition of Offspring**

There were no differences among the four groups with respect to gestation length, F(3,32)=1.32, p>0.05, with 27.8% of the rats in all 4 groups delivering on Day 22, 66.7% on Day 23 and 5.7% on Day 24. Nor were there significant differences for duration of parturition, F(3,32)=1.0, with mean durations ranging from 11.8±11.1 min for the MPO Group to 15.8±15.4 min for the MFB Group. There were no differences among the groups with respect to litter size, F(3,32)<1.0 (means ranged from 9.0±1.3 pups for the MPO Group to 13.9±1.3 for the SHAM Group); percentage of each litter born alive, F(3,32)<1.0 (means ranged from 84.7±2.7% for the ASYM Group to 92.9±3.3% for the SHAM Group); or live pup weight, F(3,32)<1.0 (means ranged from 5.4±0.2 g for the SHAM Group to 6.0±0.2 g for the MFB Group). None of the biparous rats cannibalized their young.

**Hypothalamic Neuropeptide**

Almost all the rats with MPO (n=8), ASYM (n=7), or SHAM cuts (n=13) ate all their placentas, but only two of the eight rats with MFB cuts ate all their placentas. The differences among the groups were statistically significant, F(3,32)=6.66, p<0.01. The MPO, ASYM, and SHAM Groups were not different from each other, but all were significantly more placentophagic than the MFB Group. There were no differences among the groups with respect to ingestion of donor placenta 1 hr after parturition, F(3,32)<1.0. The percentage of each of the groups that ate donor placenta were: MPO: 88%; MFB: 88%; ASYM: 100%; SHAM: 80%
Pup-Direct Maternal Behavior and Nestbuilding

Almost all the biparous rats with ASYM cuts ate all their delivered placenta, and most of them displayed immediate retrieving, crouching, and pup-picking. These results suggest that preparturitional experience protects placentaophagia and pup-directed maternal behavior from being disrupted by the abrupt change to the longitudinal system found in Experiment 1 to be necessary for their rapid onset during the first parturition (see Table 1). This does not appear to be true for nestbuilding, however, since ASYM cuts delayed the onset of nestbuilding in biparous rats just as they did in primiparous rats. This suggests that the connections between the MPO and the posterior MFB continue to play an important role in producing the rapid onset of nestbuilding, even in rats that have gained considerable maternal experience by rearing a first litter. But since most of the primiparous and biparous rats with ASYM cuts eventually did build nests, these connections appear to be involved only in promoting the rapid onset of nestbuilding at parturition.

Although MPO and MFB cuts did not affect the onset of crouching and pup-picking in biparous rats, they did produce profound effects on other behaviors. MPO cuts eliminated retrieving and nestbuilding for the entire 5-day test period, just as in primiparous. These effects were not duplicated by ASYM cuts, and therefore cannot be attributed to the disruption of a longitudinal system, suggesting that the lateral connections of the MPO continue to play an important role in mediating retrieving and nestbuilding, even in rats with maternal experience.

MFB cuts in biparous rats disrupted parturitional placentaophagia, eliminated nestbuilding for the entire 5-day test period, and produced hyperphagia on a high-fat diet. These effects were not duplicated by ASYM cuts, and were similar to the effects of MFB cuts observed in the primiparous rats of Experiment 1. It is possible that a neural system coursing through the posterior MFB but not the longitudinal system involving the MPO participates in the control of placentaophagia and nestbuilding in both primiparous and biparous rats. It remains possible, though, that the observed deficits in placentaophagia and nestbuilding may have been secondary to knife-cuts-induced alterations of ingestive behavior and activity level.

Test for Hyperphagia

As expected, biparous rats with MFB cuts were hyperphagic on all 10 days of exposure to the high-fat diet. They ate an average of 7.8% more of their body weights on each day of testing, compared with 5.9%, 6.0%, and 5.2% for rats with MPO, ASYM, and SHAM cuts, respectively, F(3,31) = 2.36, p < 0.01. Significant differences among the groups were apparent as early as Day 2, F(3,53) = 3.25, p < 0.05. By Day 10, the MFB rats averaged 120% of Day 0 weight, whereas the other groups averaged 106% of Day 0 weight.

Histological Analysis

Examination of brain sections revealed that the MPO, MFB, and ASYM cuts were similar in placement and extent to those described in Experiment 1.
TABLE I

<table>
<thead>
<tr>
<th>Behavior</th>
<th>Primiparous</th>
<th>Biparous</th>
</tr>
</thead>
<tbody>
<tr>
<td>MPO cats</td>
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<td></td>
</tr>
<tr>
<td>Placentophasia</td>
<td>Incomplete</td>
<td>Complete</td>
</tr>
<tr>
<td>Retaining</td>
<td>Never</td>
<td>Never</td>
</tr>
<tr>
<td>Crowing</td>
<td>Delayed</td>
<td>Immediate</td>
</tr>
<tr>
<td>Pup-licking</td>
<td>Delayed</td>
<td>Immediate</td>
</tr>
<tr>
<td>Nestbuilding</td>
<td>Never</td>
<td>Never</td>
</tr>
<tr>
<td>MFB cats</td>
<td></td>
<td></td>
</tr>
<tr>
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</tr>
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</tr>
<tr>
<td>Nestbuilding</td>
<td>Never</td>
<td>Never</td>
</tr>
<tr>
<td>ASYM cats</td>
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</table>

The data suggest the possibility that placentophasia is governed by two independent processes, one which involves an attraction to placenta, c simple willingness to consider placenta as an indispensable substance, and the other which shows the tendency of enthusiastic preperation of placenta, membranes, and fluids that emerge at delivery. Only this latter process was disrupted by the knife cuts that prevented the rapid onset of pup-directed maternal behavior and nestbuilding.
REFERENCES


