

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* 38(5)629-640, 1986.—Recent investigations suggest that the disruption of placentophagia, 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 placentophagia, nestbuilding, and pup-directed maternal behaviors at, and after, parturition in both primiparous and biparous rats. In primiparae, MPO cuts (a) disrupted placentophagia, (b) delayed the onset of crouching and pup-licking, and (c) eliminated retrieval and nestbuilding. MFB cuts (a) disrupted placentophagia, (b) delayed the onset of maternal behavior, and (c) eliminated nestbuilding. Asymmetrical cuts (a) disrupted placentophagia, and (b) delayed the onset of maternal behavior. In biparous rats, MPO cuts eliminated nestbuilding and retrieval. MFB cuts (a) disrupted placentophagia, and (b) eliminated nestbuilding. Asymmetrical cuts (a) delayed nestbuilding. These results suggest the involvement of a longitudinal neural system in the production of immediate pup-directed maternal behavior, placentophagia, and nestbuilding in parturient primiparae, but which is not critical for the eventual display of maternal behavior and nestbuilding in maternally naive rats, nor for the immediate onset of placentophagia and maternal behavior in maternally experienced rats.

Hypothalamus	Maternal behavior	Placentophagia	Rats	Knife cuts	Medial preoptic area
Medial forebrain bundle	MPO	MFB			

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 extensive 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 exteroceptive stimuli to the point of inducing 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 lateral preoptic area, more specifically the fibers coursing through it, may also be implicated in the control of maternal behavior. Parasagittal 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 reticular formation, and pontine central gray [5, 10, 11, 36, 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, 60]. 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-ventral tegmentum 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 supraoptic 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 [29–31].

Since the initiation of placentophagia (ingestion of the afterbirth) and infant-directed caretaking behavior occur in close temporal contiguity during or after parturition in most mammals, and since placentophagia 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 the physiological control mechanisms for placentophagia in primiparae are different from those in multiparae, and control mechanisms in virgins are different from those in parturient rats (see [22] for review). Kristal and his colleagues found that lateral hypothalamic lesions that eliminated feeding and drinking also eliminated placentophagia in nonpregnant nulliparae presented with placentas obtained from donor females, and in rats giving birth for the first time, but did not eliminate placentophagia in nonpregnant multiparae presented with donor placenta, or in rats giving birth for the second time [21,37]. Likewise, Noonan and Kristal [38] found that medial MPO lesions had no effect on placentophagia in virgins, but that such lesions did disrupt placentophagia 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 placentophagia in nonpregnant rats are different from those that regulate placentophagia in parturient rats, and that postpartum maternal behavior and placentophagia may have some common neural elements.

Since MPO lesions disrupt parturitional placentophagia as well as postpartum caretaking behavior in primiparae, and since lateral hypothalamic lesions eliminate parturitional placentophagia in primiparae, it is plausible that connections between the MPO and the lateral hypothalamus are important for the onset of both behaviors at parturition. With re-

gard to pup-directed maternal behavior, this hypothesis is strongly supported by the finding that knife cuts that sever the lateral connections of the MPO and that disrupt maternal behavior necessarily sever axons connecting the MPO and lateral hypothalamus, and by the finding of several investigators that deficits in postpartum maternal behavior are evident after lesions or knife cuts that damage the lateral hypothalamus, and after lesions or knife cuts that damage the MFB as it courses through the lateral hypothalamus [1–3, 21, 42]. With regard to parturitional placentophagia, the importance of MPO-lateral hypothalamic pathways is supported by the results from Kristal's laboratory showing that MPO lesions disrupt parturitional placentophagia as well as pup-directed maternal behavior, and that lateral hypothalamic lesions disrupt parturitional placentophagia in primiparae.

In summary, the MPO and its lateral connections are important for the initiation of placentophagia and pup-directed maternal behavior in parturient rats, for the maintenance of maternal behavior in lactating primiparous rats, for the hormonal induction of maternal behavior in hysterectomized-ovariectomized pregnant rats, and for pup-induced maternal behavior in virgin rats. The importance of the MPO and its lateral connections for placentophagia and maternal behavior in multiparae, however, has not yet been investigated. It is possible that the degree of importance differs in nulliparae, primiparae and multiparae, since the physiological mechanisms that operate to produce placentophagia and the rapid onset of maternal behavior during the first parturition may not be the same mechanisms that operate during subsequent parturitions, or that operate in virgins to produce placentophagia or pup-induced maternal behavior [6–8, 16, 21, 22, 30, 32–34, 50, 51].

The two studies presented here were designed to determine (a) whether the integrity of the longitudinal system consisting of fibers running from the MPO to the lateral preoptic area and then descending through the MFB is important for the initiation of pup-directed maternal behavior at parturition, (b) whether this system is also important for placentophagia at parturition, and (c) whether parturitional experience affects this hypothalamic control system. Accordingly, Experiment 1 addressed the hypothesis that the initiation of placentophagia and the rapid onset of nestbuilding and pup-directed maternal behavior in parturient primiparae are mediated by connections between the MPO and the MFB. This was done by comparing the effects of bilateral knife cuts on the lateral border of the MPO with bilateral knife cuts of the posterior MFB, and with knife cuts combining a unilateral MPO cut with a contralateral MFB cut (asymmetrical cuts). Those behaviors disrupted by all three types of knife cuts can therefore be considered to be controlled by a continuous functional system linking the MPO and the MFB. Use of the asymmetrical knife-cut design rests on the assumption that the connections between the MFB and the MPO must be severed bilaterally in order to produce deficits [18,46]. This assumption is supported by findings to be reported here that animals with unilateral knife cuts are not different from animals with sham knife cuts. Also, within the framework of this design, we compared the effects of the different knife cuts on each of several different behaviors characteristic of maternal female rats. Behavioral observations of all groups therefore included tests of placentophagia, nestbuilding, retrieving, crouching, and pup-licking. Finally, since Kristal has shown that the behavior of nonpregnant rats towards placenta is dichotomous—they

either eat donor placenta when first exposed to it, or they avoid it (for review, see [22])—knife 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

Subject rats ($n=45$), pup-donors, and placenta-donors were Long-Evans females, 3–6 months old, born and raised in our laboratory. Their parents 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 pretest. 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 placentophagia pretest. Donor placentas were obtained surgically from CO₂-killed, Day 21 pregnant females, then placed in small plastic vials with a few drops of physiological saline and frozen at –20°C until needed. 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 placentophage, 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 pretest. One to two weeks after the placentophagia pretest, each rat was habituated overnight to a 45×19×25-cm plastic cage containing 3 cm of coarse sawdust 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 retrieved, attacked, or ate any of the pups. Spontaneous retrievers 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 nonplacentophage status. Each rat in-

cluded as a nonplacentophage in this experiment was required to demonstrate a refusal to eat placenta in a fourth test conducted as described in the placentophagia pretest. This 1-day test occurred on a non-proestrus day of the estrous cycle 1 to 2 weeks prior to time-breeding.

Knife-cut surgery. Bilateral knife cuts were made stereotaxically in rats anesthetized with methoxyflurane (Metofane, Pitman-Moore) on the afternoon of Day 20 of pregnancy. The knife was a modified version of that described by Sclafani and Grossman [53] and consisted of a 28-ga guide cannula bent and filed at one end in such a way that a 38-ga stainless steel wire forced through the cannula extended in the direction of the curved end. For MPO 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 7.0 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 knife 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 8.0 mm below the skull surface, extending the inner wire approximately 1.5 mm toward midline, lowering the knife assembly to 9.0 mm below the skull surface, raising it to the initial point of extension, 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 MPO or MFB sham cuts were subjected to the same surgical treatments described above, except that the inner wire was not extruded. Each rat in the ASYM Group received a unilateral MPO cut and a contralateral MFB cut. Half of the rats in the ASYM-Control Group received a unilateral MPO cut and a sham MFB cut, and the other half received a unilateral MFB cut and a sham MPO cut.

Measures 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 Day 21, body weight 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 nestbuilding observations and testing. Beginning on Day 22 of pregnancy each rat was observed for parturition during frequent spot checks. At delivery, continuous observations were made to determine the incidence of placentophagia, 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 placentas in a glass dish.

Six hours after completion of delivery, each mother was weighed, the number of delivered placentas that remained uneaten 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 during the next 15 min retrieval, pup licking, and crouch-

ing over pups were noted. Afterward, any existing 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 5, 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 [14] that the elevated level of food intake characteristic of lactating females does not decline to preweaning 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 shortening (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 exposed to the high-fat diet 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 μ 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 MFB 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 subjects were run. The design was a 2 \times 3 design (Placenta Predisposition [placentophages/nonplacentophages] \times Knife Cut [MPO, MFB, SHAM]). When we determined that there were no significant main or interaction effects of placenta predisposition on placentophagia or maternal behavior, data from placentophages and nonplacentophages were pooled for all subsequent analyses. Because placenta predisposition had no effect, and because placentophages make up the large

majority of our colony, all the rats receiving ASYM cuts were placentophages.

Postoperative Food and Water Intake

Nine rats (3/15 with MPO cuts, 5/17 with MFB cuts, and 1/9 with ASYM 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 hemorrhaging 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 primiparous rats with MPO, MFB, ASYM, and SHAM cuts. An analysis of variance indicated a significant effect of surgery on food intake, $F(3,49)=3.67$, $p<0.01$, with food intakes (mean percentage of body weight) of $4.8\pm 0.7\%$; $7.7\pm 0.9\%$; $8.3\pm 1.3\%$; and $5.9\pm 0.5\%$, for MPO, MFB, ASYM, and SHAM Groups, respectively. Subsequent pairwise comparisons (all pairwise comparisons after ANOVAs were done by a Newman-Keuls test, $ps<0.05$) revealed that rats with MPO cuts ate significantly less than those with MFB or ASYM cuts, but not significantly less than the SHAM Group. The MFB and ASYM Groups did not eat significantly more than the SHAM Group. There were no significant differences in water intake among the four groups, $F(3,49)=1.16$, $p>0.05$. Mean water intake (percentage of body weight) ranged from $13.0\pm 1.0\%$ for the SHAM Group to $17.7\pm 2.7\%$ 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 74.6 ± 8.2 min for the MPO Group to 107.9 ± 23.3 min for the ASYM Group. The data on gestation length were computed 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, $F(3,49)<1.0$. Based on data gathered across all 4 groups, 15.1% of the rats delivered on Day 22, 64.15% delivered on Day 23 and 20.75% delivered on Day 24.

There were no significant differences among the four surgical groups with respect to litter size, $F(3,49)<1.0$ (means ranged from 8.9 ± 1.1 pups for the ASYM Group to 9.9 ± 1.0 for the SHAM Group); percentage of each litter born alive, $F(3,49)=2.2$, $p>0.05$ (means ranged from $54.9\pm 11.3\%$ for the MPO Group to $84.3\pm 4.8\%$ for the SHAM Group); or live pup weight, $F(3,49)=2.18$, $p>0.05$ (means ranged from 4.5 ± 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 nonplacentophage with MPO cuts, a placentophage with MFB cuts, and a nonplacentophage with MFB cuts) ate only dead pups and never attacked live pups.

Placentophagia

Delivered placentas. The percentages of groups of nonplacentophages with MPO, MFB, and SHAM cuts that ate all their delivered placentas were not significantly different from those of placentophages 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 these 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 significantly different from those of placentophages with comparable cuts, $F(1,33)<1.0$. When data from placentophages and nonplacentophages were combined, there were no significant 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: 57%.

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) nestbuilding. For each of these dependent measures, the resulting set of dichotomous data was analyzed using a 1-within, 1-between ANOVA [26] 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, $F(3,112)=17.72$, $p<0.01$, 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 MFB Group, $F(10,490)=15.48$, $p<0.01$, and in the ASYM Group, $F(10,490)=7.18$, $p<0.01$. That is, rats with MFB or ASYM cuts showed a delayed onset of retrieving; at least half of each of these groups had retrieved pups by Test 11 on Day 5 postpartum.

Crouching. Figure 1 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, $F(3,101)=11.39$, $p<0.01$. 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 three knife-cut groups, MPO: $F(10,490)=10.79$, $p<0.01$; MFB: $F(10,490)=8.13$, $p<0.01$; ASYM: $F(10,490)=9.18$, $p<0.01$. By Test 11, there were no significant differences among the four groups, $F(3,101)=2.19$, $p>0.05$.

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, $F(3,119)=13.53$, $p<0.01$. The percentages of rats in the three knife-cut groups that licked pups during this test were significantly lower than that of the SHAM Group but were not different from each other. Over the course of testing, however, there were significant increases in the percentage of rats licking pups in each of the three knife-cut groups, MPO: $F(10,490)=17.02$, $p<0.01$; MFB: $F(10,490)=14.48$, $p<0.01$;

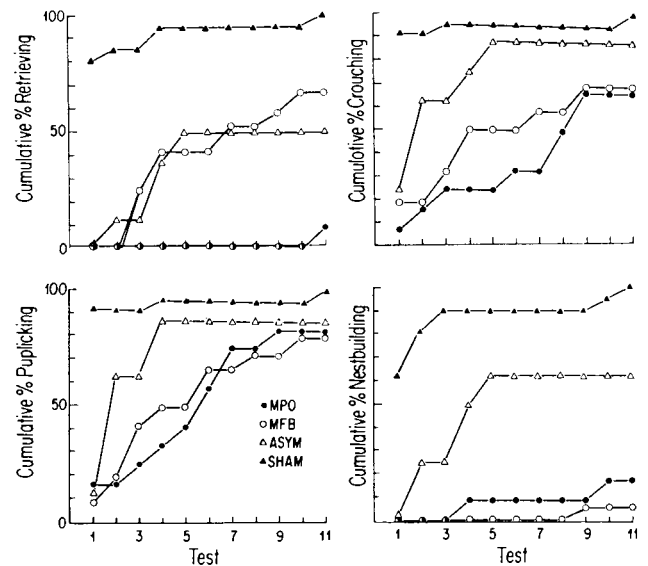


FIG. 1. Cumulative percentages of groups of primiparous rats, after knife-cut surgery, that displayed retrieving, crouching, pup-licking and nestbuilding across tests.

ASYM: $F(10,490)=8.04$, $p<0.05$. By Test 11, there were no significant differences among the groups, $F(3,119)<1.0$.

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, $F(3,111)=11.81$, $p<0.01$. 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, $F(10,490)=1.13$, $p>0.05$, nor for the MFB Group, $F(10,490)=1.70$, $p>0.05$, but did increase for the ASYM Group, $F(10,490)=10.70$, $p<0.01$. MPO or MFB cuts eliminated 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 hyperphagic 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 5.7% 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

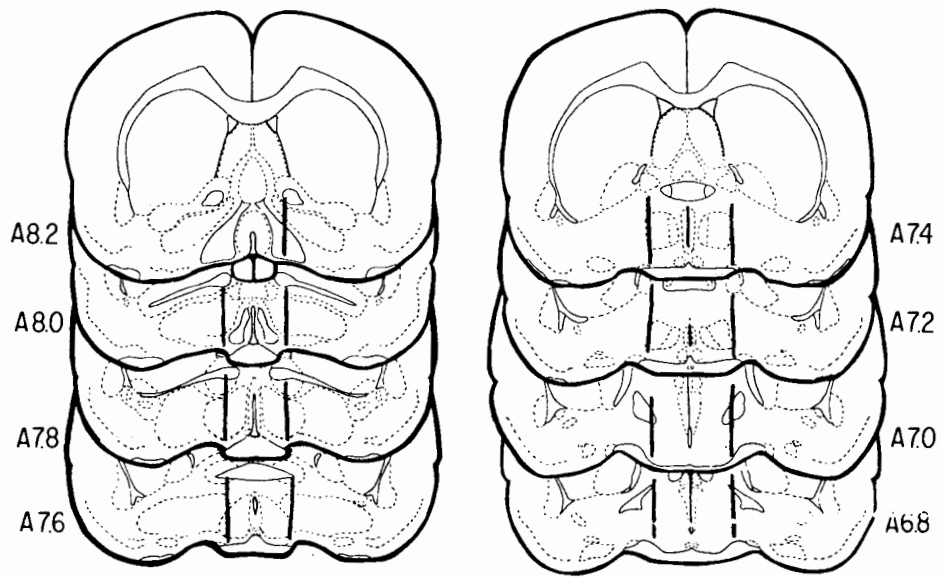


FIG. 2. Schematic representation of a typical MPO knife cut.

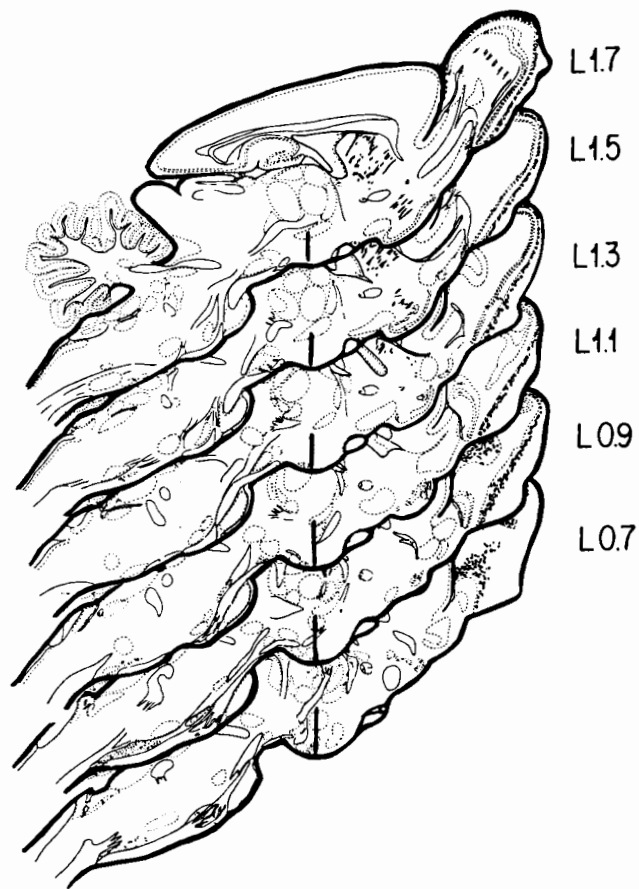


FIG. 3. Schematic representation of a typical MFB knife cut.

hypothalamic area (A 6.8–7.0), severing most of the mediolateral connections of the MPO 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 extent of the MPO (A 7.4–7.6) and extended well into the anterior hypothalamic area (A 6.6–6.8).

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 5.0 [47]. The MFB was cut bilaterally in all 12 rats from L 0.7 to 1.7 and from just ventral to the mammillothalamic tract (H -3.0) to the base of the brain. Thus, the fornix and paraformical 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 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 rat with MPO cuts that cannibalized some of its 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 placentas. However, comparison of the knife-cut damage in these rats with that in rats that did not eat all their delivered placentas revealed no relationship between knife-cut placement and parturitional placentophagia. Similarly, no relationship was found between placement of MPO cuts and the incidence of nestbuilding (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 placentas, and prevented the display of retrieving, crouching, pup-licking, and nestbuilding at 6 hr postpartum. These findings suggest that connections between the MPO 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 periparturitional behavior at delivery in primiparous rats. That the knife-cut-induced deficits in the onset of placentophagia, maternal behavior, and nestbuilding 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 placentophagia, retrieving, pup-licking, and nestbuilding 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, pup-licking, and nestbuilding. This suggests that the eventual display of maternal behavior and nestbuilding 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., pup-licking but not nestbuilding) 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 variability in 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 nestbuilding 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 lateral connections of the MPO that are important for retrieving and nestbuilding apparently are 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 nestbuilding [29–31]. Despite these long-term deficits in retrieving and nestbuilding, 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 eliminated nestbuilding 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 cuts that extend into the medial hypothalamic area produce hyperphagia [29,52]. Although the production of hyperphagia by MFB cuts casts 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 conclusions that a longitudinal system mediates the onset of parturitional behaviors; rats with ASYM cuts exhibited deficits in these behaviors but were not hyperphagic.

On the basis of observations made at 6 hr postpartum that rats with knife cuts had not eaten all their delivered placentas, 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, the 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 at pups and placentas 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 placentas still attached.

Finally, comparison of the effects of knife cuts on placentophagia in females that were either placentophages or nonplacentophages as virgins revealed that prior ingestion of placenta, 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 hypothalamic knife cuts on periparturitional 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 rats 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 placentophage as a nonpregnant nullipara, and had subsequently given birth to a litter and had raised it to weaning. One to 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 intakes (as percentage of body weight) for groups of biparous rats with MPO, MFB, ASYM, and SHAM cuts were $5.3 \pm 0.8\%$; $6.6 \pm 0.7\%$; $5.7 \pm 0.8\%$; $5.6 \pm 0.3\%$, respectively. The mean water intakes (percentage of body weight) for these groups were: MPO: $18.0 \pm 3.7\%$; MFB: $12.8 \pm 2.0\%$; ASYM: $14.4 \pm 1.9\%$; SHAM: $14.1 \pm 1.2\%$. There

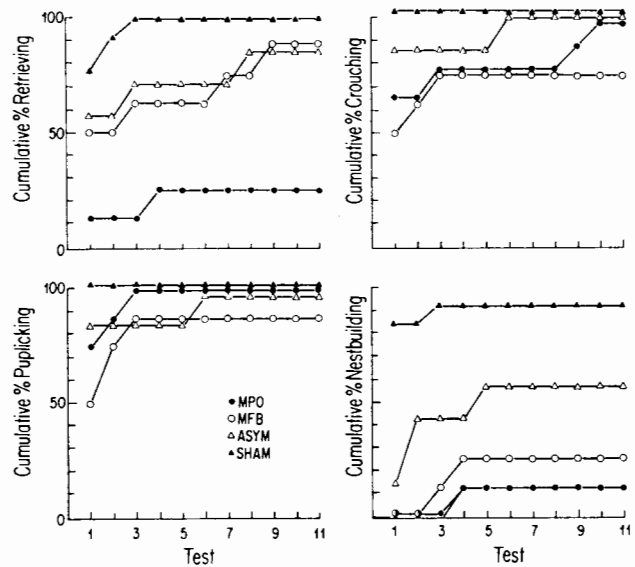


FIG. 4. Cumulative percentages of groups of biparous rats, after knife-cut surgery, that displayed retrieving, crouching, pup-licking, and nestbuilding across tests.

were no significant differences among the groups in food intake, $F(3,32) < 1.0$, or water intake, $F(3,32) < 1.0$.

Timing of Parturition and Condition of Offspring

There were no differences among the four groups with respect to gestation length, $F(3,32) = 1.52$, $p > 0.05$, with 27.8% of the rats in all 4 groups delivering on Day 22, 66.7% on Day 23 and 5.5% on Day 24. Nor were there significant differences for duration of parturition, $F(3,32) < 1.0$, with mean durations ranging from 113.8 ± 11.1 min for the MPO Group to 153.8 ± 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 $84.7 \pm 12.7\%$ for the ASYM Group to $92.9 \pm 3.3\%$ 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 MPO Group). None of the biparous rats cannibalized their young.

Placentophagia

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: 85%.

Pup-Directed Maternal Behavior and Nestbuilding

Retrieving. Figure 4 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, $F(3,61)=4.32, p<0.01$. The percentages of rats in the MFB, ASYM, and SHAM Groups that retrieved on this test were significantly greater than that of the MPO Group, but were not different from each other. At least half the rats with MFB or ASYM cuts retrieved pups on Test 1. By contrast, only 1 of 8 rats with MPO cuts retrieved on this test, and only 2 of 8 had retrieved pups by the end of the 5-day test period.

Crouching. Figure 4 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, $F(3,65)=4.55, p<0.01$. Fewer rats in the MPO and MFB Groups crouched over pups on this test than in the ASYM or SHAM Groups. Despite the differences among the groups, however, it should be noted that at least half the rats in each of the groups crouched over pups on Test 1, and by Test 2 the differences among the groups were not statistically significant.

Pup-Licking. Figure 4 shows the cumulative percentage of rats in each group that licked pups. There were significant differences among the groups on Test 1, $F(3,79)=7.40, p<0.01$. The percentages of rats in the MPO, ASYM, and SHAM Groups that licked pups on this test were significantly greater than that of the MFB Group ($p_s<0.05$), but were not significantly different from each other. However, note that 50% of the rats in the MFB Group did lick pups on Test 1, and 75% were doing so by Test 2. There were no significant differences among the groups on Test 2, $F(3,79)<1.0$.

Nestbuilding. Figure 4 shows the cumulative percentage of rats in each group that built nests. The effects of knife cuts on nestbuilding in biparous rats were similar to the effects seen in primiparous rats. There were significant differences among the groups on Test 1, $F(3,49)=9.92, p<0.01$. 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. MPO or MFB cuts eliminated nestbuilding for the entire 5-day test period, but ASYM cuts only delayed the onset of nestbuilding; more than half the rats in the ASYM Group had built nests by Test 11.

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% 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, $F(3,31)=12.36, p<0.01$. Significant differences among the groups were apparent as early as Day 2, $F(3,51)=3.25, p<0.05$. By Day 10, the MFB rats averaged 130% of Day 0 weight, whereas the other groups averaged 108% 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.

DISCUSSION

Almost all the biparous rats with ASYM cuts ate all their delivered placentas, and most of them displayed immediate retrieving, crouching, and pup-licking. These results suggest that prior parturitional experience protects placentophagia and pup-directed maternal behavior from being disrupted by damage 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-licking 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 primiparae. 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 placentophagia, 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 placentophagia and nestbuilding in both primiparous and biparous rats. It remains possible, though, that the observed deficits in placentophagia and nestbuilding may have been secondary to knife-cut-induced alterations of ingestive behavior and activity level.

GENERAL DISCUSSION

At the time of parturition, the female rat ingests the delivered placentas, amniotic fluid, and fetal membranes, and begins to provide maternal care in the form of pup-licking, nursing, nestbuilding, and retrieval of stray pups back to the nest. The present experiments were designed to examine the effects of hypothalamic knife cuts, with and without previous parturitional experience, on these components of periparturitional behavior. The results of these experiments showed that (a) the medial preoptic area and the posterior medial forebrain bundle, due to the nature of the connections between them, constitute a functional, longitudinal neural system that is involved in producing the rapid onset of both the ingestive and caretaking components of periparturitional behavior in the primiparous rat. (b) The effects of severing these connections are probably due to the direct disruption of a neural system specifically involved in producing the rapid onset of these behaviors, since the critical cuts that interfered with their onset did not produce alterations in

TABLE 1
SUMMARY OF EFFECTS OF KNIFE CUTS ON PLACENTOPHAGIA, ONSET OF MATERNAL BEHAVIORS, AND NESTBUILDING IN PRIMIPAROUS AND BIPAROUS RATS

Behavior	MPO cuts		MFB cuts		ASYM cuts	
	Primiparous	Biparous	Primiparous	Biparous	Primiparous	Biparous
Placentophagia	Incomplete	Complete	Incomplete	Incomplete	Incomplete	Complete
Retrieving	Never	Never	Delayed	Immediate	Delayed	Immediate
Crouching	Delayed	Immediate	Delayed	Immediate	Delayed	Immediate
Pup-licking	Delayed	Immediate	Delayed	Immediate	Delayed	Immediate
Nestbuilding	Never	Never	Never	Never	Delayed	Delayed

feeding and drinking behavior, did not influence the timing of onset or duration of parturition, and did not affect the viability of offspring. (c) Although this longitudinal system is involved in producing the rapid onset of periparturitional behavior, it does not appear to be necessary for the gradual onset of pup-directed maternal behavior and of nestbuilding. Many of the primiparae with asymmetrical cuts, which severed the longitudinal system bilaterally, displayed full maternal responsiveness after 5 days of continuous exposure to foster pups. (d) The lateral connections of the medial preoptic area, but not those which pass through the posterior medial forebrain bundle, appear to be essential for the elicitation by foster pups of maternal retrieving and nestbuilding. After 5 days of continuous exposure to pups, primiparae with medial preoptic area cuts licked and crouched over pups, but did not retrieve pups or build nests. (e) Prior ingestion of placenta, as a nonpregnant nullipara, did not prevent the disruption of parturitional placentophagia by hypothalamic knife cuts. (f) Prior parturitional experience protected placentophagia and pup-directed maternal behavior, but not nestbuilding, from disruption after damage to the longitudinal system found to be necessary for the rapid onset of these behaviors during the first parturition. (g) The role of this system in controlling nestbuilding in biparous rats is limited to the production of its rapid onset, just as it is in primiparous rats. (h) The lateral connections of the medial preoptic area continue to play an important role in mediating retrieving and nestbuilding, even in rats with prior parturitional experience.

It is important to note the parallels between the present findings, that a longitudinal neural system is involved in the production of immediate maternal responsiveness in primiparous but not in biparous rats, and previous work indicating the importance of prepartum hormonal changes for the rapid onset of maternal behavior in primiparous but not in biparous rats [32,34]. It is possible that the effects of knife cuts on periparturitional behaviors in primiparous rats were not produced directly, by disrupting a neural system specifically involved in producing rapid onset, but were produced indirectly by preventing the changes in gonadal hormone secretions thought to be necessary for immediate maternal responsiveness. Although this latter possibility cannot be ruled out, it seems unlikely, because eliminating the prepartum estrogen increase or progesterone decline would have influenced the time of onset or duration of parturition [4,9].

A reasonable alternative explanation for the parallels be-

tween the effects of knife cuts and the effects of hormonal manipulations is that the cuts disrupted a neural system that ascends through the MFB and acts in synergy with estrogen on medial preoptic area neurons to produce the rapid onset of maternal responsiveness in parturient primiparae, but that neither the functional integrity of this neural system nor the presence of estrogen is necessary during the second parturition. This suggestion is consistent with previous findings that the medial preoptic area is the neural site on which estrogen acts to facilitate the onset of maternal behavior (for review, see [13]), and with evidence from electrophysiological experiments demonstrating both direct and indirect input from fibers in the posterior medial forebrain to cells in the medial preoptic area [48]. On the other hand, these results do not preclude the possibility that the behavioral effects were produced by a disruption of MPO efferents [42].

Nestbuilding was the only component of periparturitional behavior that failed to appear immediately in biparous rats with asymmetrical cuts. The reason for this is unclear, but one possibility is that asymmetrical cuts disrupted hypothalamic mechanisms of temperature regulation [27] and thereby affected maternal nestbuilding indirectly [41].

One of the aims of the present experiments was to determine whether the neural systems mediating placentophagia overlap with those mediating maternal behavior. The results of Experiment 1 suggest that a longitudinal neural system does, in fact, participate in the control of both pup-directed maternal behavior and placentophagia; hypothalamic cuts that prevented the rapid onset of maternal behavior also reduced the likelihood that a rat would eat all her delivered placentas. It is important to note, however, that cuts did not eliminate parturitional placentophagia, nor did they affect the proportion of rats that ate donor placenta presented one hour after delivery. Primiparae with cuts apparently found placenta to be a palatable substance, since all but one ate some of their delivered placenta, but they lacked the motivation to eat all.

These data suggest the possibility that placentophagia is governed by two independent processes, one which involves an attraction to placenta, a simple willingness to consider placenta as an ingestible substance, and the other which produces the complete and enthusiastic consumption of placentas, 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.

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