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Uterine Distention Facilitates the Onset of Maternal Behavior in Pseudopregnant but not in Cycling Rats[1]

GARY C. GRABER AND MARK B. KRISTAL[2]

Department of Psychology, State University of New York at Buffalo, Buffalo, NY 14226

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GRABER, G. C. AND M. B. KRISTAL. *Uterine distention facilitates the onset of maternal behavior in pseudopregnant but not in cycling rats.* PHYSIOL. BEHAV. 19(1) 133–137, 1977. – The latency to onset of maternal behavior toward foster pups was examined in maternally-naive female rats treated either with uterine distention, a sham procedure, or no uterine manipulation. Uterine distention was achieved by intrauterine injection of hypertonic saline. The treatments were applied to either cycling, Day 10 pseudopregnant, or Day 11 pseudopregnant-decidualized virgins. The latency to onset of maternal behavior for both pseudopregnant groups was significantly shorter than that for the nonpseudopregnant group, when uterine distention was applied. The results suggest that uterine distention during pregnancy (during high progesterone level) may bring about both pregnancy termination (delivery) and the almost immediate maternal behavior seen at parturition, by the same hormonal mechanism.

Uterine distention Maternal behavior Pseudopregnancy Deciduoma Uterus Parturition

UTERINE distention that arises from fetal development is a conspicuous feature of mammalian pregnancy. It promotes contractility and growth of the uterus and is very likely involved in the hormonal dynamics of pregnancy-termination (parturition) by exerting a luteolytic effect through the possible release of prostaglandin-$F_{2\alpha}$ from the uterus [2, 3, 5, 17]. The consequent decrease in progesterone results in disinhibition of uterine contractions, leading to expulsion of the fetuses. The natural termination of pregnancy is accompanied by the almost immediate onset of maternal behavior.

Despite these observations, uterine activity has not received much attention as a critical factor in the onset of maternal behavior at parturition. This oversight appears to have been the result of demonstrations that premature relief of uterine distention does not facilitate, and prolonged uterine distention does not interfere with, the development of maternal responsiveness [4]; and that elimination by cesarian section of normal delivery of young at term, does not alter the onset of maternal responsiveness [8]. However, although these studies are addressed to critical issues such as the effect of the relief from uterine distention, and the effect of delivery experience, they do not demonstrate the importance, or lack thereof, of the increase in uterine distention prior to delivery.

Since uterine distention is involved in producing hormonal changes just prior to parturition, changes that have been implicated in the facilitation of the onset of maternal responsiveness in the rat [6, 7, 12, 15, 16, 19, 20], we decided to examine this chain of events more directly by examining the effect of uterine distention on the initiation of maternal behavior in rats during prolonged corpus luteum function and elevated progesterone (pseudopregnant) and during ovarian cyclicity (nonpregnant). The use of pseudopregnant rather than pregnant animals was designed to have the female in a condition of prolonged corpus luteum function without having to control for changes in fetoplacental physiology that would occur in pregnant animals.

Uterine distention was accomplished by intrauterine injections of hypertonic saline. This technique was modified from the intraamniotic hypertonic saline injection technique applied to women to induce abortion [2]. In nonpregnant uteri, the injection not only distends, but, because of the hypertonicity of the distending medium, may also prolong the duration of distention by sequestering water. Although the distending and sequestering effects of hypertonic saline injections cannot be separated from the irritation that probably occurred, the procedure will herein simply be referred to as uterine distention.

Three groups of rats, nonpregnant, pseudopregnant, and pseudopregnant-decidualized, were treated with either uterine distention, a sham procedure, or nothing, and observed for their response to foster pups, allowing for a 3 × 3

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[2] Send reprint requests to: Mark B. Kristal, Department of Psychology, SUNY at Buffalo, 4230 Ridge Lea, Buffalo, NY 14226.

factorial analysis. Pseudopregnant rats were treated on Day 10 of pseudopregnancy, when progesterone level is known to be high, and 24-hr before it is expected to decline sharply [9,10]. The effect of the presence of a deciduoma in pseudopregnancy is to prolong the duration of pseudopregnancy to about 16 days. Therefore the pseudopregnant-decidualized group was used to control for the imminence of the normal termination of pseudopregnancy and the return to cyclicity: pseudopregnant-decidualized rats were treated with uterine distention on Day 11 of pseudopregnancy (6 days after deciduoma-induction), when progesterone level was known to be high, but 5 days before it was expected to decline sharply [10, 13, 14].

Subsequently, an additional group of pseudopregnant rats was given uterine distention on Day 11 of pseudopregnancy, as a control for the day of pseudopregnancy on which uterine distention was applied, in order to permit comparison of the behavior of this group to that of the Day 10 pseudopregnant females that had received uterine distention.

Our expectation was that since the hypothesized route of influence of uterine distention on maternal behavior was the lowering of elevated progesterone level by luteolysis produced by $PGF_{2\alpha}$-release from the distended uterus, then uterine distention would have no effect on the induction of maternal behavior in nonpregnant (nonpseudopregnant) rats. On the other hand, since lowering of progesterone (pseudopregnancy-termination or pregnancy-termination) is the proposed critical event, the effect of uterine distention on pseudopregnant and pseudopregnant-decidualized females was expected to be facilitory. Furthermore, if uterine distention, rather than depending on natural pseudopregnancy-termination, simply requires a progesterone-dominated system on which to act, then the magnitude of the facilitation produced by uterine distention should be the same for both the pseudopregnant and pseudopregnant-decidualized groups. Finally, uterine distention was expected to be effective in facilitating the Day 10 pseudopregnant rats, but not the Day 11 pseudopregnant rats, since the progesterone levels are expected to have already begun to decline in the latter group.

METHOD

Animals

The study was conducted on 105 virgin Long-Evans rats (Charles River Breeding Laboratories), that were 120–150 days of age when tested. Each female, except during maternal-behavior testing, was housed individually in 19 × 24 × 18-cm wire-mesh cages. Food (Charles River Rat/Mouse/Hamster Formula) and water were available ad lib. The entire laboratory was subjected to a 14-hr on/10-hr off light cycle (lights on at 6:00 a.m. EST); the rooms were illuminated with red light during the dark phase. Noncycling females and 3 spontaneous retrievers were eliminated from the animal pool prior to the formation of experimental groups.

Pup-donors were time-bred Long-Evans females, 12–15 weeks of age, born and raised in our laboratory. Donor cages with litters were maintained in a room separated from that of the experimental animals.

Procedure

Grouping. Nonpseudopregnant rats received either uterine distention (NPs/UD), a sham procedure (NPs/S), or no uterine manipulation (NPs/NO). Initially, both the NPs/UD and NPs/S Groups had been evenly divided into 3 subgroups, one receiving the operation during estrus, one during proestrus, and one during diestrus, to examine the possibility of an interaction between the manipulation and stage of the estrous cycle. When no differences between stages of the cycle were found, the subgroups were pooled, yielding an n of 18 rats/group. The NPs/NO Group (n = 29) resulted from the reconsolidation of 3 subgroups that proved not to be significantly different from one another: an intact-cycling subgroup (n = 10), an ovariectomized subgroup (n = 9), and a sham-ovariectomized, cycling subgroup (n = 10).

The second reproductive-condition group consisted of rats on Day 10 of pseudopregnancy that received either uterine distention (Ps/UD; n = 6), the sham procedure (Ps/S; n = 6), or no uterine manipulation (Ps/NO; n = 6).

The third reproductive-condition group consisted of rats that were decidualized on Day 5 of pseudopregnancy, then received either uterine distention (Ps+D/UD; n = 7), the sham procedure (Ps+D/S; n = 5), or no uterine manipulation (Ps+D/NO; n = 4), on Day 11 of pseudopregnancy.

A subsequent group of 6 rats receiving uterine distention on Day 11 of pseudopregnancy (no deciduoma) was run to compare with the Day 10 Ps/UD group, to assess the effect of uterine distention when applied after the decline of progesterone level had already begun.

Uterine distention. Uterine distention was applied under ether anesthesia. The uterine horns were exposed by means of bi-ventrolateral laparotomy, and 1 ml of hypertonic saline (20% NaCl, w/v) was injected into the uterine lumen through a 0.5-in, 26-ga hypodermic needle that had been threaded into the lumen from the tubal end of the uterus. After completion of the injection, the needle was withdrawn with the tip held against the antimesometrial aspect of the lumen to prevent damage to the uterine vascular supply, and to provide uniform damage to the endometrium. The puncture site was held momentarily to facilitate closure, then inspected for leakage, which was usually minimal. Animals in the S groups (sham procedure) were similarly treated, except that (a) the syringe was closed, and (b) the needle was filled with normal saline to prevent air from entering the uterine lumen. All uterine manipulations were performed between 9:00 and 11:00 p.m.

Pseudopregnancy. Pseudopregnancy was induced in 40 rats by the application of electrical stimulation to the cervix at approximately 2:00 p.m. on the day of estrus. The stimulation was applied with a 5-mm diameter glass probe (lubricated with mineral oil) which held 2 stainless steel wires (0.010 in., insulated except at the tips) a distance of 2 mm apart [1]. Three 10-sec trains of 1-msec rectangular pulses, 25 V, 200 Hz, were delivered at random intervals within a 50-sec period. The stimulus was generated by a Grass S8 stimulator and monitored on a Tektronix 502A oscilloscope. This method produced a 95% rate of success of pseudopregnancy-induction.

After the cervical stimulation procedure, daily vaginal smears were obtained; a rat was considered to be pseudopregnant only if she manifested a diestrus vaginal smear on the fourth day after cervical stimulation, and continued to show persistent diestrus through the day of the experiment [21].

Deciduoma induction. Deciduomas were induced in 16 pseudopregnant rats, on Day 5 of pseudopregnancy, by

surgical disruption of the uterine endometrium [21]. The pseudopregnant rats were anesthetized with ether and the uterine horns exposed by bi-ventrolateral laparotomy. A 1.5-in, 20-ga, burred hypodermic needle was inserted into each horn through the tubal end of the uterus. The burr was dragged along the antimesometrial aspect of the uterus several times before the needle was withdrawn. Daily vaginal smears were taken thereafter until the day of the experiment. Verification of deciduoma induction was obtained by visual inspection during the UD or S procedures performed on these animals on Day 11 of pseudopregnancy. Only those rats in which deciduomas were verified remained in the experiment. All the deciduomatized rats that showed persistent diestrus until Day 11, when they were given the UD or S treatment, bore a deciduoma. Only those females in Ps+D/NO Group that showed persistent diestrus to Day 11 were tested; although they were not visually examined for deciduoma, the probability that they all had deciduomas was exceedingly high.

Maternal-behavior testing. Two or three hr before the uterine manipulation was performed, each rat was placed in a transparent plastic 'maternity' cage (15 × 24 × 35 cm) which contained coarse sawdust for bedding, and which was fitted with a wire-grill top containing both food and water. The maternity cages were located in a testing room that was separated from both the colony and the surgery. After the uterine manipulation was performed, the rats were returned to these cages for recovery and for maternal behavior tests.

After the period of recovery from surgery (at about midnight), 6 foster pups (approximately 5 days of age) were introduced into the maternity cage. The criteria for the maternal-behavior test were taken from Rosenblatt [11]; all 4 were required: the rat (a) must have built a nest which contains all the pups; (b) must be observed to crouch or assume a nursing posture over the pups, in that nest; (c) must be observed to lick the perianal region of the pups; and (d) must be observed to retrieve all 6 pups to the nest within 15 min after they were scattered around the cage.

The first observation period (the first scattering of pups) began 30 min after the introduction of the foster litter to the maternity cage. Thereafter, observations (begun by scattering the pups) were made at 8:00 a.m. and 8:00 p.m. until the animal was rated maternal, or until the testing period was finished. When the criteria for maternal responsiveness were satisfied, the rat was assigned a latency score representing the number of days of exposure to pups. Rats that did not become maternal by the end of the 8-day test period were arbitrarily assigned a latency score of 8 days.

The resulting data, except for the post-hoc Day 11 Pseudopregnant-UD Group, were analyzed as a 3 × 3 factorial design analysis of variance, using the unweighted means technique for unequal n's. Pairwise comparisons were made using the Newman-Keuls test. The comparison between the Ps/UD Group and the Day 11 Pseudopregnant-UD Group was made using a *t*-test.

RESULTS

The data for all groups are presented in Table 1. The ANOVA performed on 3 reproductive conditions (nonpseudopregnant, NPs; Day 10 pseudopregnant, Ps; pseudopregnant-decidualized, Ps+D) and 3 treatments (uterine distention, UD; sham procedure, S; no uterine manipulation, NO) revealed a significant effect of treatment, $F(2,90) = 12.26$, $p<0.001$, a significant interaction between treatment and reproductive condition, $F(4,90) = 6.27$, $p<0.001$, but no significant effect of reproductive condition, $F(2,90) < 1$.

Newman-Keuls analyses of group means indicated that the main effect of reproductive condition was obscured by the interaction between treatment and reproductive condition. The form of this interaction is illustrated in Fig. 1. The Newman-Keuls analysis of the effect of treatment over

TABLE 1

LATENCY TO ONSET OF MATERNAL BEHAVIOR ($\bar{X}$ DAYS ± SEM) OF RATS IN THREE REPRODUCTIVE CONDITIONS TREATED EITHER WITH UTERINE DISTENTION, A SHAM PROCEDURE, OR NO UTERINE MANIPULATION (8-DAY TEST)

Group	n	Latency	% Maternal in 2 Days	% Maternal in 8 Days
Nonpseudopregnant				
NPs/UD*	18	4.18 ± 0.51	17	89
NPs/S*	18	3.32 ± 0.63	33	89
NPs/NO†	29	6.74 ± 0.31	00	48
Pseudopregnant				
Ps/UD	6	1.63 ± 0.63	67	100
Ps/S	6	6.90 ± 0.51	00	50
Ps/NO	6	5.08 ± 0.76	00	83
Day 11 UD	6	3.41 ± 1.16	50	83
Pseudopregnant + Deciduoma				
Ps+D/UD	7	2.01 ± 0.45	57	100
Ps+D/S	5	5.84 ± 0.86	00	20
Ps+D/NO	4	3.67 ± 0.75	25	100

*Group evenly divided among females receiving UD during proestrus, estrus, and diestrus (no differences between subgroups).

†Group contains 10 intact, cycling virgins, 9 ovexed virgins, and 10 sham-ovexed virgins (no differences between subgroups).

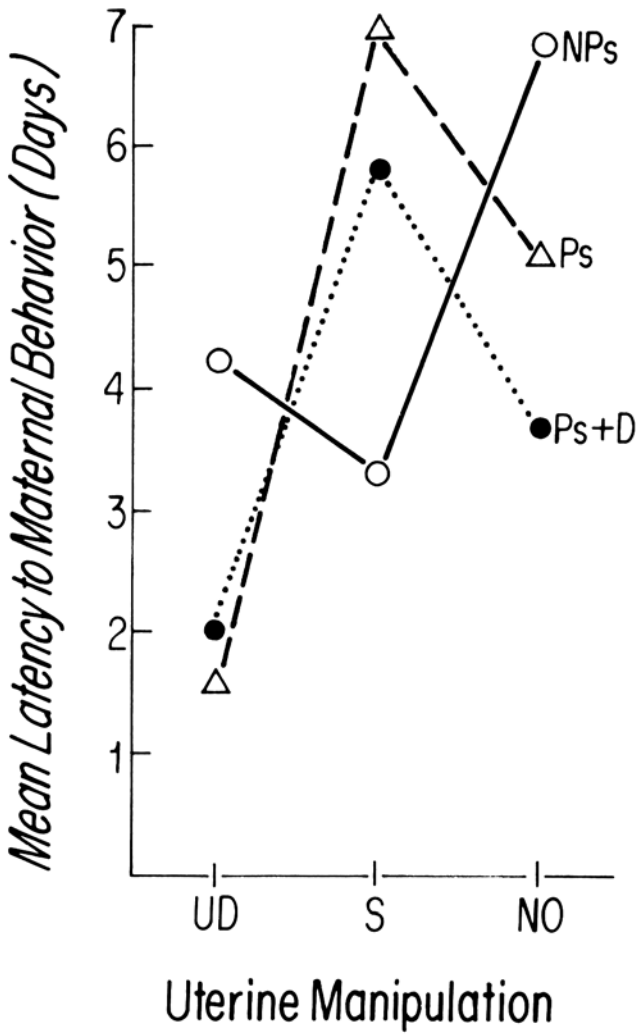

FIG. 1. The interaction between treatment and reproductive condition on the latency to manifest maternal behavior. Treatments: UD = uterine distention; S = a sham procedure; NO = no uterine manipulation. Reproductive conditions: NPs = nonpseudopregnant; Ps = pseudopregnant; Ps+D = pseudopregnant–decidualized.

reproductive conditions indicated that at $p<0.05$, the latencies of the Ps/UD and Ps+D/UD Groups were significantly shorter than that of the NPs/UD Groups, and were not significantly different from one another; the Ps/S and Ps+D/S Groups showed significantly longer latencies than did the NPs/S Group, and were not significantly different from each other; and the Ps+D/NO Group latency was significantly shorter than that of the NPs/NO Group, but that the Ps/NO Group was not significantly different from either. The Newman-Keuls analysis of the effect of reproductive condition over treatments indicated that at $p<0.05$, the latencies for NPs/UD and NPs/S were not significantly different from one another, but both were significantly shorter than that of NPs/NO; the Ps/UD latency was significantly shorter than those of both Ps/S and Ps/NO, but that the latter 2 groups were not significantly different from one another; and that the Ps+D/UD latency was significantly shorter than those of both Ps+D/S and Ps+D/NO, but that of Ps+D/S was significantly longer than Ps+D/NO. Therefore, the data indicate that uterine distention did facilitate the onset of maternal behavior in both groups of pseudopregnant rats, but not in the nonpseudopregnant rats. Furthermore, the uterine surgical procedure, whether real or sham, slightly facilitated the onset of maternal responsiveness in nonpseudopregnant rats, relative to nonpseudopregnant controls, but had nowhere near the facilitory effect of real uterine distention in pseudopregnant rats. In fact, the sham procedure had a slight inhibitory effect on maternal behavior in pseudopregnant rats, when they were compared with pseudopregnant rats with no uterine manipulation.

The results also indicate that uterine distention is effective in facilitating the onset of maternal behavior in pseudopregnant rats because they are pseudopregnant, and not because of the nearness to the end of pseudopregnancy, since the latencies of the Ps/UD and Ps+D/UD Groups were not different.

The mean latency for the Day 11 Psuedopregnant/UD Group fell between the value for the Ps/UD Group and that for the Nps/UD Group (see Table 1), and was not significantly different from either (Day 11 vs Ps/UD: $t(10) = 1.75$, $p>0.10$; Day 11 vs NPs/UD: $t(22) = 0.60$, $p>0.50$). It should be noted however, that a mean latency of 3.41 days, as manifested by the Day 11 Pseudopregnant/UD Group, is not considered to represent a rapid onset of maternal behavior.

DISCUSSION

The results show that uterine distention, defined as intrauterine injection of hypertonic saline, facilitates the onset of maternal behavior in pseudopregnant, but not in nonpseudopregnant rats. These results are consonant with the hypothesis that uterine distention requires a progesterone-dominated system on which to act, because the route of influence of uterine distention is through removal or termination of progesterone domination. Our results fit very nicely with the evidence that (a) uterine distention does bring about the termination of progesterone domination by producing luteolysis, at the end of pregnancy [2, 3, 5, 17], and that (b) pharmacological termination of progesterone-domination (pseudopregnancy) facilitates the onset of maternal behavior in rats [18]. However, whether uterine distention actually rapidly terminates pseudopregnancy, and, if so, whether it does so by luteolysis brought about by $PGF_{2\alpha}$-release, are important steps in the logical sequence that need to be tested directly.

The moderate facilitation of the onset of maternal behavior in both nonpseudopregnant UD- and S-treated rats, when compared to the difference between UD and S treatment in both groups of pseudopregnant rats (see Fig. 1), indicates a possible interaction between some nonspecific aspect of the surgical procedure, and the reproductive condition of the animal. A likely candidate for the nonspecific factor is the application of ether anesthesia immediately before testing; the slight but statistically significant decrease in latency manifested by rats so treated, suggests a hormonal basis for the phenomenon, and warrants further examination.

Finally, it should be noted that methodologically, the uterine distention technique is an efficient surgical-mechanical method for inducing a rapid onset of maternal behavior, without having to introduce exogenous hormones or pharmacological agents into the system of the female.

In summary, the research has demonstrated that the distention of the uterus, during pseudopregnancy, significantly reduces the latency to display maternal behavior in maternally-naive rats. A major implication of this finding is that distention of the uterus late in pregnancy brings about one set of physiological (hormonal) changes which account both for pregnancy-termination (initiation of delivery) and for the extremely rapid onset of maternal behavior observed at delivery.

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