

PLACENTA ON PUPS' SKIN ACCELERATES ONSET OF MATERNAL BEHAVIOUR IN NON-PREGNANT RATS

BY MARK B. KRISTAL, JEFFREY F. WHITNEY & LAWRENCE C. PETERS

Department of Psychology, State University of New York at Buffalo, 4230 Ridge Lea Road, Amherst, N. Y. 14226, USA

Abstract. Previous research has indicated that virgin rats (*Rattus norvegicus*) behave maternally (sensitize) more rapidly if kept in close proximity with pups. Since both parturient rats and a large percentage of virgin rats avidly consume placenta, we tested whether placenta and amniotic fluid, applied to the skin of the stimulus pups, would draw the female adults into closer contact with the pups and therefore hasten the onset of maternal behaviour. The results indicated that the procedure indeed shortened the maternal sensitization latency. Furthermore, this effect was not due to the wetness of the pups, to the presence of placenta in the cage, or to the adults having previously ingested placenta. Other attractive ingestibles applied to the pups' skin produced an intermediate, but not significant, shortening of the maternal sensitization latency.

When maternally naive adult rats give birth, they behave maternally toward the neonates virtually immediately. When maternally naive virgins are housed continuously with foster pups (concoaveation), they too behave maternally toward the pups, but not immediately. The latency for this maternal behaviour (maternal sensitization latency) to appear is of the order of days (Wiesner & Sheard 1933; Cosnier & Couturier 1966; Rosenblatt 1967). The length of the maternal sensitization latency has been shown to be affected not only by hormonal and neural manipulations (see Lamb 1975; Rosenblatt et al. 1979; Slotnick 1975 for review), but also by environmental manipulations. Terkel & Rosenblatt (1971) demonstrated that if adult virgins and pups are forced into close proximity during concoaveation by housing them in small cages, maternal sensitization latencies are shorter. It appears then that maternal sensitization is dependent, at least in part, on the interaction of intensity and duration of exposure to pups. We would expect, therefore, that factors that promote non-maternal, non-harmful behaviour of the female adult toward the pups should have a facilitating effect on the induction of maternal behaviour during concoaveation.

Birch (1956) suggested that mother rats are attracted to pups at delivery by substances on the skin of the pups that are reminiscent of their own anogenital areas, to which they become intensely attracted as a consequence of pregnancy. Whether or not substances on the skin of the pups are reminiscent of the mothers' anogenital attractants, there are substances on or associated with the skin of neonates that are extremely attractive to rat mothers and, in fact, to

most other non-human mammalian mothers: foetal membranes, placenta, and amniotic fluid (Slijper 1960; Lehrman 1961; Kristal 1973; Kristal & Graber 1976). The elimination of the ingestion of placenta at parturition by a previously induced taste aversion did not lead to obvious deficits in maternal behaviour (Engwall & Kristal 1977), but the effect of licking amniotic fluid from the skin of each rat pup and of consuming foetal membranes was neither investigated nor controlled for. That maternal behaviour appears readily toward cleaned foster pups in Caesarean-delivered rats (Moltz et al. 1966) is also not evidence against a role for attractants on the skin of the pups. In that study (a) the stimulus pups had already been licked and cleaned by their own mothers, and may therefore have been more attractive in their own right than younger, uncleaned neonates that may be attractive only because of the material on their skin; (b) tests were conducted during the postpartum period, when other mechanisms that promote the rapid onset of maternal behaviour were apparently still in effect; and (c) detailed analysis of the mother's behaviour during the first few minutes of exposure to pups, when the effect of the attractiveness of pups' skin may be maximal, was not conducted.

Although the attraction of the mother to the skin of the pup may have a beneficial effect on the pup and on the mother's responsiveness to the pup in the immediate postpartum period, the effect is difficult to assess at that time. It would seem more productive to test the effect of attractive substances on the skin of the pups (a) in isolation from the hormonal factors contributing to the rapid onset of maternal behaviour at

parturition, and (b) using a procedure in which the maternal behaviour is induced slowly, so as to allow for observation of facilitation.

A large percentage (approximately 45%) of the virgin female rats in our laboratory avidly and immediately ingest donor placenta (mixed with amniotic fluid) the first time it is presented to them (Kristal & Graber 1976). By the response of the rats, it appears that the attractiveness of placenta to the placenta-eating virgins is very similar to the attractiveness of the afterbirth to parturient rats. We decided to test, therefore, whether these virgin placenta-eaters (placentophages) would show reduced maternal sensitization latencies if the pups presented to them were repeatedly smeared with placenta and amniotic fluid.

Methods

The subjects were 115 virgin female Long-Evans (hooded) rats (*Rattus norvegicus*), approximately 120 days old, weighing approximately 250 g. Forty-nine of the subjects were purchased (Charles River Breeding Laboratories) at eight weeks of age; the rest, born and raised in our laboratory, were the offspring of purchased parents. During the experiment, the rats were housed individually in 46 × 24 × 21-cm clear Plexiglas cages containing about 2 cm of coarse sawdust for bedding. The cage tops contained food pellets (Charles River Rat/Mouse/Hamster Formula 3000) and a water bottle, for ad libitum access. The entire laboratory was maintained on a 14:10 day:night cycle, with the lights-on phase beginning at 0600 hours (EST).

When each rat was approximately 10 weeks old, a daily examination of cell types in the vaginal smear was begun in order to verify normal ovarian cyclicity. When the rats completed two normal oestrous cycles they were given two pretests. The first pretest was used to determine the behaviour of each rat toward donor placenta. Using procedures described elsewhere (Kristal & Graber 1976), each rat was given one 15-min exposure to donor placentas on each of three consecutive days. These placentas (and some associated amniotic fluid) had been obtained surgically from CO₂-killed donors on day 21 of pregnancy, and had been frozen and stored at -20 C. Immediately prior to use, the placentas were warmed rapidly to 37 C and proffered in untippable glass dishes. The response of female rats tested this way is clearly dichotomous: either they eat donor placenta enthusiastically, usually on the first

presentation, or they actively avoid it and will not eat it at all (Kristal & Graber 1976). Non-eaters do ingest placenta during delivery of their own pups, however. Those rats that were determined by this pretest to be placentophages underwent a second pretest. Each placentophage was offered a litter of five stimulus pups, all the same age, which were from three to eight days old. Females that retrieved the pups during the 15-min exposure period were judged to be spontaneous retrievers, and were removed from the study. The proportion of spontaneous retrievers in rats is small, and varies among strains (approximately 4% in our Long-Evans virgins); spontaneous retrievers are routinely excluded from subject pools in which the behavioural dependent variable is the rate of onset of maternal responsiveness (e.g. Terkel & Rosenblatt 1971). The resulting pool of 115 subjects, therefore, comprised only placentophagic non-retrievers.

To test for maternal behaviour, each rat was presented daily at 0900 hours with a fresh, uniform-aged litter of five three- to eight-day-old pups. The rat was then observed for the next hour. She was rated as maternal if she did all of the following: (a) retrieved all the pups to nest site within the first 15 min; (b) licked the pups vigorously, particularly in the anogenital area; and (c) crouched or hovered over the pups in a nursing posture (Rosenblatt 1967). The pups remained in the cage until replaced by another litter the next day. On the first day of continuous exposure to pups a check for rapid onset of maternal behaviour was made at 1500 hours. On each test day a retrieval test was conducted at 2100 hours by scattering the pups about the cage and observing the adult's response for 15 min.

Various substances were applied to the skin of the stimulus pups to test for the effect of the attractiveness of those substances on the rate of maternal sensitization. Each adult was presented with stimulus pups of only one of seven treatment groups. The sensitization rates for females given treated pups were compared with those of females given untreated pups in a normal concaveation procedure (baseline group, $N = 18$). The first substance tested was placenta/amniotic fluid (referred to hereafter simply as placenta). Prior to presentation to the female at 0900 hours, each pup was coated with a mixture of chopped placenta and amniotic fluid. The mixture was made from donor placentas that had been removed and stored in a manner described above. The tissue and associated fluid of five placentas

were used to coat each litter of five pups. All pups in all groups were handled with disposable plastic gloves. The observer always knew which substance was on the pups' skin.

To control for the presence of placenta in the cage, a group was tested (pups + dish placenta, $N = 10$) in which untreated pups were presented simultaneously, each day, together with a dish containing five placentas and associated fluids. To control for the effect of ingesting placenta, a group of females was tested that had been given five placentas (with associated fluid) in a dish for five consecutive days prior to the first day of pup exposure (pups after dish placenta, $N = 24$).

A group was then tested to control for the possibility that the effect of placenta on the skin was really an effect of vocalizations or other stimuli emitted by cool, wet pups. Physiological saline, rather than placenta, was applied to the skin of the pups presented to this group of rats (saline on pups, $N = 7$).

The final controls were for the presence of bloody rat tissue other than placenta, and for palatable substances other than placenta. To control for bloody rat tissue, liver, which had been removed from non-pregnant female rats and stored and presented in a procedure identical to that used with placenta, was applied to pup skin instead of placenta (liver on pups, $N = 12$). To control for the presence of an attractive, palatable substance, rather than for placenta specifically, one group was tested with pups coated with saccharin (Sucaryl®, 1.21% saccharin solution) and one with pups coated with a demonstrably attractive mash (e.g. Teitelbaum et al. 1969) made of chocolate chip cookies (Chips Ahoy®) and milk (cookie on pups, $N = 12$).

The twice-daily observations were continued until the rat behaved maternally or until 12 days

of testing had elapsed. Rats that had not behaved maternally by the end of the twelfth day were assigned a latency of 13 days.

Results

Since several of the groups contained rats that did not show maternal behaviour by the end of the 12-day test, and were therefore assigned an arbitrary upper-limit score, all group comparisons were made with Mann-Whitney U tests. A summary of the results is presented in Table I.

The shortest mean latency exhibited was for the group proffered pups smeared with placenta (placenta on pups, 3.85 ± 0.56 days, $\bar{X} \pm \text{SEM}$). The rate of sensitization in this group was significantly faster than that of the baseline group (baseline, 6.31 ± 0.80 days; $U = 131$, $z = 2.31$, $P = 0.018$). By comparing the second fastest group with baseline (cookie on pups, 4.58 ± 0.62 days; $U = 83.5$, $z = 1.04$, $P > 0.10$) and the slowest group with baseline (pups after dish placenta, 7.35 ± 0.70 days; $U = 243$, $z = 0.69$, $P > 0.20$), it was clear that the only group to differ significantly from baseline was that in which placenta was smeared on the pups.

The latency of the group in which liver was smeared on the pups' skin (5.00 ± 0.74 days) did not differ from that of the group in which placenta was smeared on the skin ($U = 111.5$, $z = 1.25$, $P > 0.10$). This suggests that cookie and liver on the skin may have had a slight facilitating effect that resulted in intermediate values between the effect of placenta-smeared pups and the effect of untreated pups.

Regardless of the effect of the treatment on maternal sensitization, the rats in the placenta, cookie, and liver groups began licking off the pups within the first 1 or 2 min after presentation. Duration of licking episodes was not recorded.

Table I. Effect of Exposure to Different Pup Treatments on the Latency for Virgin Rats to Manifest Maternal Behaviour

Treatment*	<i>N</i>	$\bar{X} \pm \text{SEM}$ days latency	Range	Median days latency	% maternal in two days	% maternal in test
Placenta on pups†	25	3.85 ± 0.56	0.25-12.0	3.5	20	100
Cookie on pups**	12	4.58 ± 0.62	2.0- 9.0	4.3	17	100
Liver on pups††	12	5.00 ± 0.74	2.0-10.0	4.5	8	100
Baseline	18	6.31 ± 0.80	1.5-13.0	6.0	6	89
Saccharin on pups	7	6.71 ± 1.35	3.5-13.0	5.0	0	86
Saline on pups	7	6.79 ± 1.13	1.0-10.5	7.5	14	100
Pups + dish placenta	10	7.10 ± 1.09	2.0-12.0	8.0	10	100
Pups after dish placenta**	24	7.35 ± 0.70	2.5-13.0	7.0	0	83

* Treatments are presented in order of increasing latency.

† Significantly different from baseline, Mann-Whitney U , $P < 0.02$.

** Not significantly different from baseline, Mann-Whitney U , $P > 0.05$.

†† Not significantly different from placenta on pups, Mann-Whitney U , $P > 0.05$.

The rats presented with a dish of placenta immediately began eating the placenta at each presentation. The rats presented with saline- or saccharin-swabbed pups, like those in the baseline group, ignored the pups totally for the first few presentations.

The frequency of cannibalism of pups was quite low, and was not recorded systematically in all groups. The highest incidence, however, appeared to be among rats exposed to placenta-smearred pups: of 15 rats observed for cannibalism, 3 cannibalized pups on two different occasions.

Discussion

Mother rats seem strongly attracted to the after-birth and foetal fluids at parturition, in that they usually immediately consume the placenta and membranes and lick amniotic fluid from the skin of the pups (Wiesner & Sheard 1933; Lehrman 1961; Rosenblatt & Lehrman 1963). A large proportion of virgin female rats also find placenta and amniotic fluid extremely attractive (Kristal & Graber 1976). Since the induction rate of maternal responsiveness in virgin rats has been found to be influenced by the intensity and duration of exposure to pups (Cosnier & Couturier 1966; Rosenblatt 1967; Terkel & Rosenblatt 1971), we designed the present study to test whether placenta, applied to the pups' skin, would increase the rate of maternal sensitization, presumably by hastening or increasing contact between the adult and the pups. In this way the attractiveness of the substances on the skin would be counteracting the apparent aversiveness of pups to the naive non-pregnant female (Terkel & Rosenblatt 1971).

The results of the study indicate that, indeed, placenta and amniotic fluid applied to the skin of the stimulus pups facilitates the onset of non-hormonally based, or pup-exposure induced, maternal behaviour in naive virgin rats. Furthermore, the effect was not merely due to the presence of placenta in the cage, to recent ingestion of placenta, or to the effect of pups with cool, wet skin. The results did suggest, however, that other attractive ingestible substances may also facilitate sensitization, but that the influence of those substances is more subtle than that of placenta and amniotic fluid.

Although applying placenta and amniotic fluid to the skin of the stimulus pups in the concave paradigm produced a relatively modest effect (reducing the maternal sensitization latency from 6.31 to 3.85 days), the procedure did pro-

duce immediate pup-licking. It is tempting to speculate that the immediate attraction of the female to the skin of pups during delivery would have a much more potent effect on the initiation of maternal behaviour because of the hormonal milieu of the mother at that time. In other words, it is possible that the attractiveness of the substances on and associated with the skin of the pup induces immediate contact between the adult female and the pups. The brevity of the interval between this initial contact and the onset of full-blown maternal responsiveness, however, would then be a function of the extent to which the female's hormonal condition approximates to the optimal condition seen at delivery.

Acknowledgments

This study was supported by grant BNS76-04316 awarded to M. B. K. by the National Science Foundation. We thank Seymour Axelrod for his advice regarding preparation of the manuscript. We would like to acknowledge the participation of Lolita T. Wang in an earlier version of the study.

REFERENCES

- Birch, H. G. 1956. Sources of order in the maternal behavior of animals. *Am. J. Orthopsychiat.*, **26**, 279-284.
- Cosnier, J. & Couturier, C. 1966. Comportement maternel provoqué chez les rattees adultes castrées. *C. R. Soc. Biol.*, **160**, 789-791.
- Engwall, D. B. & Kristal, M. B. 1977. Placentophagia is modifiable by taste aversion conditioning. *Physiol. Behav.*, **18**, 495-502.
- Kristal, M. B. 1973. Effects of lateral hypothalamic lesions on placentophagia in virgin, primiparous and multiparous rats. *J. comp. physiol. Psychol.*, **84**, 53-62.
- Kristal, M. B. & Graber, G. C. 1976. Placentophagia in nonpregnant rats: influence of estrous cycle stage and birthplace. *Physiol. Behav.*, **17**, 599-605.
- Lamb, M. E. 1975. Physiological mechanisms in the control of maternal behavior in rats: a review. *Psychol. Bull.*, **82**, 104-119.
- Lehrman, D. S. 1961. Hormonal regulation of parental behavior in birds and infrahuman mammals. In: *Sex and Internal Secretions* (Ed. by W. C. Young), pp. 1268-1382. Baltimore: Williams & Wilkins.
- Moltz, H., Robbins, D. & Parks, M. 1966. Caesarean delivery and maternal behavior of primiparous and multiparous rats. *J. comp. physiol. Psychol.*, **61**, 455-460.
- Rosenblatt, J. S. 1967. Nonhormonal basis of maternal behavior in the rat. *Science*, **156**, 1512-1514.
- Rosenblatt, J. S. & Lehrman, D. S. 1963. Maternal behavior of the laboratory rat. In: *Maternal Behavior in Mammals* (Ed. by H. L. Rheingold), pp. 8-57. New York: John Wiley.

- Rosenblatt, J. S., Siegel, H. I. & Mayer, A. D. 1979. Progress in the study of maternal behavior in the rat: hormonal, sensory, and developmental aspects. In: *Advances in the Study of Behavior*, Vol. 10 (Ed. by J. S. Rosenblatt, R. A. Hinde, C. Beer & M. C. Busnel), pp. 225-311. New York: Academic Press.
- Slijper, E. J. 1960. Die Geburt der Säugetiere. In: *Handbuch der Zoologie*, Vol. 8 (Ed. by J. G. Helmcke, H. V. Lengerken & D. Starck), pp. 1-108. Berlin: Walter de Gruyter.
- Slotnick, B. M. 1975. Neural and hormonal basis of maternal behavior in the rat. In: *Hormonal Correlates of Behavior*, Vol. 2 (Ed. by B. E. Eleftheriou & R. L. Sprott), pp. 585-656. New York: Plenum Press.
- Teitelbaum, P., Cheng, M. F. & Rozin, P. 1969. Development of feeding parallels its recovery after hypothalamic damage. *J. comp. physiol. Psychol.*, **67**, 430-441.
- Terkel, J. & Rosenblatt, J. S. 1971. Aspects of non-hormonal maternal behavior in the rat. *Horm. Behav.*, **2**, 161-171.
- Wiesner, B. P. & Sheard, N. M. 1933. *Maternal Behaviour in the Rat*. London: Oliver & Boyd.

(Received 26 November 1979; revised 25 February 1980;
MS. number: A2406).