First Suckling Response of the Newborn Albino Rat: The Roles of Olfaction and Amniotic Fluid

Abstract. *Washing the nipples of anesthetized parturient rats virtually eliminated nipple attachment by their young. Normal attachment was induced only by painting the washed nipples with a distillate of the nipple wash, parturient-mother saliva, or her amniotic fluids. Reinstatement was not achieved by coating the washed nipples with the saliva of virgin females eating the same diet, the parturient mother's urine, isotonic saline, amyl acetate, or vanilla extract. These experiments also provide behavioral evidence for olfactory function in the newborn albino rat.*

Because suckling is a vital, prominent, and enduring behavior of altricial mammals, the study of its controls and their alterations during ontogeny are of interest to behavioral scientists, pediatricians, and clinicians. We have recently demonstrated that a scent must be present on the nipples of anesthetized albino rat mothers in order for nipple attachment to occur in their preauditory and previsual young (2 to 12 days of age) (*1*). Washing the anesthetized mother's nipples virtually eliminated nipple attachment. Attachments could be reinstated only by returning a distillate of the wash or of the saliva of deprived siblings to the washed nipples (*1*).

Having discovered that attachment is, in part at least, induced by a specific substance deposited on the nipples by the rats themselves, we sought to resolve the paradox of the infant albino rat's very first nipple attachment. We evaluated three hypotheses: (i) initial attachment is not under olfactory control, that is, newborn pups might attach to washed as well as unwashed nipples; (ii) a specific substance, which elicits suckling in the newborn, is either secreted around the nipple or applied there by the mother; and (iii) attachment might be elicited by a wide range of volatile substances. We now report that both the saliva of the newly parturient mother and her birth fluids contain substances that induce the initial nipple attachment in infant albino rats. This demonstration also provides behavioral evidence for olfactory capacity in albino rats within the first few hours after birth.

We conducted two experiments. In one, 84 pups from seven litters (Sprague-Dawley) were evenly distributed among six treatment groups: fresh, wash, amniotic fluid, mother's saliva, mother's urine, and virgin female's saliva. Birth was witnessed and the entire litter removed after all of the pups were cleaned but before the mother could settle over the litter to initiate suckling. The mother was anesthetized (2 ml of Equithesin per kilogram of body weight), and the pups were divided (two pups per litter per condition) (*2*). The first group was then given their initial suckling experience on their freshly anesthetized dam. Each pup in this group was assigned a nipple and lightly held and positioned by the experimenter until its snout established contact with the assigned teat. Latency to attach was recorded. Any pup which failed to attach within 2 minutes was assigned a latency score of 120 seconds.

The nipples of the anesthetized mother were then subjected to chemical lavage (*1*). A Teflon tube was lowered over a nipple and a gentle vacuum was applied. A 3:2 solution of methylene chloride and 95 percent ethanol was pipetted around the base of the tube, and it circulated around the nipple's surface until the tube was removed, and the increased airflow delivered the solution to a collecting tube (*3*). To prevent pups in the last group from being overly deprived, only four of the mother's nipples were used in these tests, and they were the only nipples rigorously washed. The nipples were rewashed between each test substance and after each attachment to provide a comparable stimulus array. After ample time had passed to assure that all the solvents used for the wash had evaporated from the mother's nipples (usually 30 minutes), the second group of pups was assigned nipples and given their first opportunity to suckle.

After this test the nipples were in turn coated (*4*) with an aliquot of mother's saliva (*5*), amniotic fluid (*6*), mother's urine (*7*), and virgin female's saliva (*5*). These substances were selected because rats extensively groom their vaginal and mammary regions during parturition, thereby coating the nipples with saliva and birth fluids (*8*). Pups in four of the seven litters were tested in pairs; in three, they were tested individually. The fresh and wash conditions were always tested first. Test order for the other four conditions was randomized between replications.

In the second experiment, we tested 96 pups from eight litters. The litters were tested in pairs; only one mother in

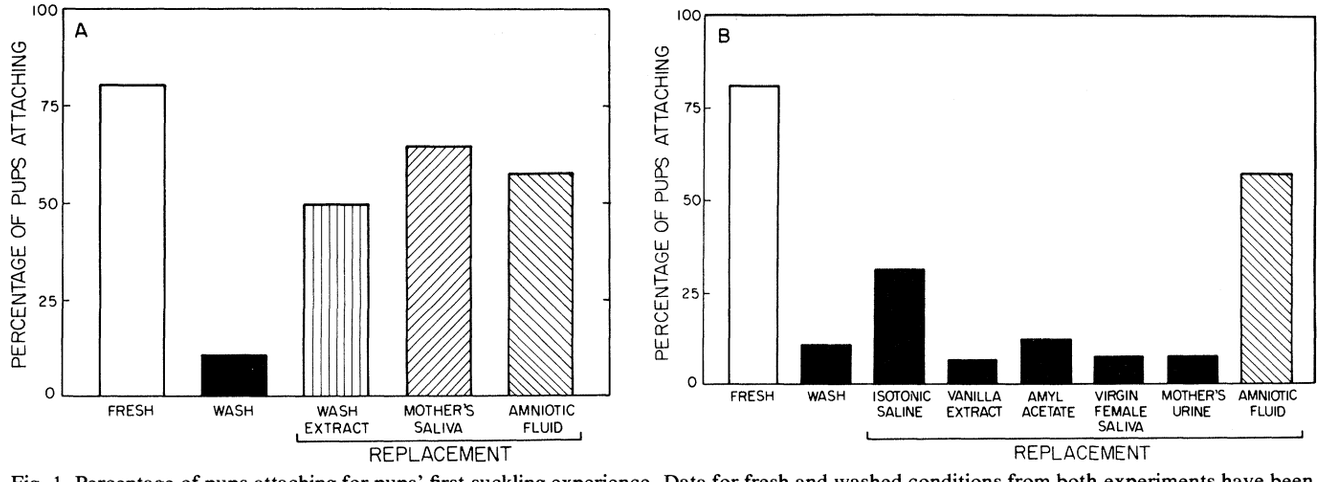

Fig. 1. Percentage of pups attaching for pups' first suckling experience. Data for fresh and washed conditions from both experiments have been combined for graphic presentation although the statistical comparisons were from the separate experiments. (A) Conditions: on the fresh mother; after nipple wash; and after a distillate of the wash, amniotic fluid, or parturient mother's saliva was painted on the nipples. (B) Conditions: on the fresh mother; after nipple wash; and after replacement with virgin female saliva, parturient mother's urine, isotonic saline, amyl acetate, or vanilla extract. Effect of amniotic fluid replacement is included for comparison.

each pair was anesthetized, so half the pups were tested on their natural mother while the other half were not. We observed no differences in attachment latencies between pups suckling on the natural or surrogate mothers. The pups were also distributed among six treatment conditions: fresh and wash (as above) and with amyl acetate (mixed isomers), isotonic saline, vanilla extract, or nipple wash extract painted onto the washed nipples (*9*).

Hypothesis (i), concerning the necessity of a volatile substance on the nipple for eliciting first suckling, was assessed by comparing nipple attachment to washed versus fresh nipples. Hypothesis (ii), regarding a particular substance, was evaluated by comparing performance on washed nipples versus those "reconstituted" with parturient maternal saliva or amniotic fluid. Hypothesis (iii), concerning the range of effective stimuli, was evaluated by determining attachment on nipples coated with various substances. For clarity, the data presentation highlights the tested hypothesis. Actual statistical comparisons were made within each experiment proper.

The fresh and wash data from the two experiments combined supports the hypotheis that removing a scent from the teats of parturient mothers interferes with nipple attachment by their young (Fig. 1A) [$z = 5.69$, $P < .001$ (*10*)]. Mean attachment latency increased from 65 to 116 seconds ($t = 6.39$, d.f. = 58, $P < .001$). Returning an extract of the wash increases attachment (Fig. 1A) [extract versus wash: $z = 3.40$, $P < .001$; versus fresh: $z = 1.43$, not significant (N.S.)] and reduces mean latency to 87 seconds [Scheffé test (*11*); extract versus wash: $P < .05$; versus fresh: N.S.].

Although washing the mother's nipples severely disrupted the suckling behavior of her newborn pups, this response was not the result of a residual aversive odor. Pups in the aversive odor control group (*3*) responded like the pups tested on the freshly anesthetized dam (aversive control versus fresh: $z = 1.51$, N.S.; versus wash, $z = 5.50$, $P < .0001$). Indeed, aversive reactions were never observed in any of the suckling tests. We therefore conclude, with regard to hypothesis (i), that some soluble substance must be on or near the nipple in order for suckling to occur. We believe that olfaction rather than taste is the major factor in this discrimination. Of the 115 pups that failed to attach on their first suckling test (both experiments) only 18 were observed to have mouthed or licked the nipples (*12*).

Both amniotic fluid and mother's saliva reinstated suckling to prewash levels (Fig. 1A) (amniotic fluid versus wash: $z = 2.37$, $P < .02$; maternal saliva versus wash: $z = 2.71$, $P < .01$; amniotic fluid versus fresh: $z = 1.67$, N.S.; maternal saliva versus fresh: $z = 1.31$, N.S.). We do not know whether maternal saliva per se can elicit suckling or if its efficacy is due to the amniotic fluid ingested during delivery and cleaning of the newborn. Thus, with regard to hypothesis (ii), substances other than the distillate of the wash removed from the mother can elicit suckling when placed on the nipple. That those substances are actively spread on the mammary region by the parturient mother provides added biological significance to the present findings.

Not all novel substances can elicit nipple attachment. None of the replacement substances (except amniotic fluid, whose effect is added for comparison) improved performance beyond the washed condition, and each differed significantly from the fresh (Fig. 1B) (*13*). Thus, nipple moisture per se is not a sufficient condition for attachment. In particular, neither mother's urine, which is presumably spread over the mammae during the mother's active grooming, nor the saliva of virgin females eating the same diet reinstated suckling. With regard to hypothesis (iii), therefore, this experiment, though by no means exhaustive, begins to satisfy the criterion for specificity and demonstrates that the initial attachment is not elicited by just any moist, volatile substance coating the nipple.

It appears that the mother produces, and probably applies to the nipple, a chemical cue that aids in the initiation of the first suckling experience. The likelihood that the cue is smelled by the newborns suggests that albino rats, within a few hours of parturition have and use some olfactory capacity. Finally, our discovery that amniotic fluid, which is swallowed by mammalian fetuses (*14*), can elicit suckling after parturition may further our understanding of putative prenatal influences on mammalian behavior.

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References and Notes

1. M. H. Teicher and E. M. Blass, *Science* **193**, 422 (1976); E. M. Blass, M. H. Teicher, C. P. Cramer, J. P. Bruno, W. G. Hall, *J. Comp. Physiol. Psychol.*, in press. Independent verification of the wash effect, by using a slightly different procedure, has been provided by M. A. Hofer, H. Shair, and P. J. Singh [*Physiol. Behav.* **17**, 131 (1976)]. P. J. Singh and M. A. Hofer [*Neurosci. Abstr.* **2**, 163 (1976)] have also shown that the rat mother's milk fails to reinstate suckling to washed nipples.
2. After removing the litter and during testing, the pups were kept in a wire basket (25 by 15 by 9 cm) lined with paper towel and the soiled bedding of the parturient dam. The basket was warmed to nest temperature by keeping it on a heating tray. Between tests (during the wash procedure) the pups were housed in Styrofoam cups (10 cm in diameter by 5 cm high) in a water bath, which maintained a warm, humid ambience similar to actual nest conditions. Four pups were housed in each Styrofoam container.
3. A control group ($N = 12$) was also tested for which the solvents were applied to their mother's ventrum and nipples (six drops per nipple, 7.5 ml over ventrum) without vacuum aspiration to assure that any alteration in suckling was due to the removal of the natural scent and not due to the deposition of an aversive odor. Likewise, nipple temperature was monitored between tests, and when necessary, it was returned to control levels by gentle warming with a heat lamp.
4. Each set of substances was applied to the nipples with a new Pasteur pipette. Aqueous solutions (isotonic saline, saliva, urine, and amniotic fluid) were applied and allowed to sit for 5 minutes, after which they were patted with a paper tissue until they were nearly dry. Substances in organic solvents (nipple wash and vanilla extracts) were allowed to dry fully so that the volatile solvents would be removed.
5. Saliva samples were obtained from either the pups' own mothers or from another newly parturient dam and an adult virgin female of comparable age and ingesting the same diet. Each rat was placed under anesthesia (Equithesin, 2 ml per kilogram of body weight). A glass pipette was inserted into the rat's mouth and saliva was aspirated with a rubber bulb. Small volumes of water were added to the oral cavity and this was aspirated as well. Between 0.2 and 0.3 ml of a foamy saliva solution were eventually gathered from each rat.
6. Amniotic fluid was obtained by cesarean section of etherized mothers early on day 21 of gestation. The uterus and the ovaries were ligated, removed, and slit. Fluid was collected in a 5-ml syringe. Two separate samples of amniotic fluid were used in this study, and only two litters were tested with the fresh fluid. The samples were stored in a refrigerator and studied for as long as 40 days after collection. Fresh fluid appeared to be more effective than older samples. Therefore, our measure of efficacy for amniotic fluid is probably overly conservative.
7. Urine was collected in a glass pipette after suprapubic pressure was applied to the anesthetized mother.
8. J. S. Rosenblatt and D. S. Lehrman, in *Maternal Behavior in Mammals*, H. L. Rheingold, Ed. (Wiley, New York, 1963).
9. The wash extract was obtained by vacuum distilling the wash solution at 30°C (Büchi Rotovapor R) to remove the solvents. Test order was partially constrained in the sense that the fresh and wash conditions were always tested first and the extract condition was always tested last. The saline, vanilla, and amyl acetate tests were randomized.
10. J. L. Bruning and B. L. Kintz, *Computational Handbook of Statistics* (Scott, Foresman, Glenview, Ill., 1968), pp. 199–201.
11. G. Keppel, *Design and Analysis: A Researcher's Handbook* (Prentice-Hall, Englewood Cliffs, N.J., 1973).
12. In addition, rats 5 and 10 days of age, which did not suckle a washed nipple, did so when an annulus of saliva was painted around but not on the nipple (that is, the gustatory cues were not changed).
13. Comparison with the washed condition: virgin female's saliva, $z = 0.61$; mother's urine, $z = 0.61$; isotonic saline, $z = 1.81$, $P > .07$; amyl acetate, $z = 0.61$; vanilla extract, $z = 0.00$ (except where noted, $P > .50$). All of these substances differed significantly from the fresh condition ($z = 4.16, 4.16, 2.85, 3.90, 4.28$, respectively; all $P < .005$). Scheffé tests on the latency scores support the same conclusions.
14. R. M. Bradley and C. M. Mistretta, *Physiol. Rev.* **55**, 352 (1975).
15. Supported by NSF grant BMS-01460 and NIH grant AM 18560 to E.M.B. and by predoctoral fellowship MHO 7138-01 from the National Institute of Mental Health to M.H.T.

5 July 1977