Short- and Long-Term Effects of Various Milk-Delivery Contingencies on Sucking and Nipple Attachment in Rat Pups

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Varied milk delivery contingencies differentially affect sucking in rat pups as measured by jaw-muscle electromyographic activity. In Experiment I we found that 11–13-day-old pups sucked more frequently when receiving tiny intermittent pulses of milk than when receiving no milk, and continued to suck at a high rate for at least 30 min after cessation of milk delivery. In subsequent experiments we found that pups 13–18 days of age engaged in more frequent sucking if receiving milk continuously rather than intermittently, and that this increased rate of sucking persisted for at least 24 hr after cessation of milk delivery. Pups given experience with continuous milk delivery from 14 to 18 days of age also spent significantly more time attached to the nipple at 19 days of age than did pups in control groups. It was concluded that experience with different milk delivery schedules can affect subsequent sucking and nipple attachment behavior.

Several investigators have demonstrated that 2-week-old rats traverse runways and mazes more efficiently when reinforcement consists of sucking a nipple which provides milk rather than sucking a nipple which does not (Letz, Burdette, Gregg, Kittrell, & Amsel, 1978; Kenny, Stolhoff, Bruno, & Blass, 1979). We are now finding that the pup's behavior while on the nipple is also affected by milk delivery. Eleven- to thirteen-day-old pups engage in more frequent sucking while receiving milk than do pups which receive no milk (Brake, Sager, Sullivan, & Hofer, 1982). Rat pups are also capable of acquiring conditioned preferences for previously aversive olfactory stimuli when those stimuli are paired with milk delivery (Brake, 1981). These findings suggest that suckling rat pups might learn when and how to feed as a function of when and how they have been fed in the past.

In this series of experiments we begin to test the hypothesis that specific milk delivery schedules have both immediate and long-term effects on sucking and nipple-attachment behavior. In the 1st experiment we explore the short-term effects of milk

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delivery on sucking. In the 2nd we examine the effects that different milk-delivery schedules have on sucking from 1 day to the next. In the 3rd we attempt to determine whether several days' experience with different milk-delivery schedules affects sucking or nipple attachment behavior at the usual age of weaning.

Experiment I

The results of our initial investigations of sucking in rat pups suggested that milk delivery might be an important factor in the control of sucking. Sucking frequency decreases if pups are allowed to suckle in the absence of milk for 30 min or more (Brake & Hofer, 1980; Brake, Wolfson, & Hofer, 1979). Further, the frequency of sucking increases immediately following a naturally occurring milk-ejection (Shair, Brake, & Hofer, 1982), and pups allowed continuous access to milk while attached to the nipple of an anesthetized dam engage in more sucking than pups allowed to suckle in the absence of milk (Brake et al., 1982). In this experiment, we wished to determine whether brief exposure to milk delivery might affect the frequency of sucking even after milk delivery is discontinued.

Method

Subjects

The subjects were 10 male and 10 female 11-13-day-old rat pups. The pups were born of Marland Farm Wister dams in the animal cage facility of Montefiore Hospital. Dams and litters were housed in 40 × 40 × 24-cm plastic terraria in temperature- and humidity-controlled rooms. The light/dark regimen was 12:12 hr with light onset at 7:00 a.m. Litters were culled to 9 pups on Day 3 (day of birth was counted as zero). Food and water were continuously available.

Design

Two groups were run, each consisting of 5 males and 5 females drawn from an average of 6 different litters. Pups in both groups were allowed to suckle an anesthetized, nonlactating dam for 2 hr, but 1 group received pulses of commercially available half-and-half (Queen's Farms) for 30 min during the middle of the sucking test, and 1 group did not.

Procedure

Separation and surgery. Sucking was monitored by recording jaw-muscle (digastric) electromyographic activity (Brake et al., 1979). Twenty hours prior to testing, pups were removed from their mothers and implanted with EMG recording electrodes of Teflon-coated silver wire (Medwire), .25 mm in diameter (see previous article). Pups that were to receive milk (half-and-half) during testing were also fitted at this time with a posterior tongue cannula of PE 10 tubing (Clay-Adams) according to the procedure described by Hall and Rosenblatt (1977). Following surgery, pups were individually placed in small plastic tubs which were then enclosed in a warm (33°C), moist (50-70% relative humidity) incubator (Forma Scientific Diurnal Growth Chamber) for the duration of their deprivation periods.

Suckling test. Electrode leads were connected to a Grass Model 7 polygraph and the pup placed with an anesthetized (urethane, 2 g/kg), nonlactating dam in a 30 X 15 X 15-cm plastic tub. Both rows of nipples were accessible. Dams were checked for milk before and after each pup's test. If milk was detected in any nipple, another dam was substituted or the pup's data described. Pups were allowed to suckle 1 at a time for 2 hr. Neither group of pups received milk during the 1st hr. During the 1st 30 min of the 2nd hr, 1 group of pups received a tiny (.005 ml) pulse of milk every 20-25 sec via the tongue cannula. A total of 90 pulses were administered, with pups receiving an average of .45 ml of milk over the 30 min. The remaining group of pups continued to suckle in the absence of milk. During the final 30-min period neither group of pups received milk. Jaw-muscle EMG was monitored and recorded throughout the entire 2 hr.

Results and Discussion

EMG Scoring

EMG records were independently scored by 2 trained raters. Raters were able to identify 2 basic types of muscle activity. We have described these types of sucking elsewhere (see preceding article) as being either brief intermittent bursts (arrhythmic sucking) or prolonged continuous bursts (rhythmic sucking; see Fig. 1). Interrater reliability in identifying each of these types of episodes ranged from 85 to 95%. We know from past (Brake et al., 1979) and ongoing work that these muscle patterns are indeed representations of arrhythmic and rhythmic intraoral negative pressure waves (sucking).

Group Differences

ANOVA performed on the pretreatment data revealed a significant decrease in the amount of sucking displayed over time [$F(1,18) = 16.37$, $p < .01$], but no differences in sucking rate between the 2 groups (Fig. 2). The decrease in sucking frequency of about

Fig. 1. Representative EMG tracings. Line A is an example of arrhythmic sucking (B = burst, T = treadle). Line B is an example of rhythmic sucking.

Fig. 2. Upper panel: Mean frequency of all types of sucking (sec/min of time attached to the nipple). Lower panel: Mean frequency of rhythmic sucking (sec/min of time attached to the nipple). The 30-min treatment phase is not presented. (X refers to frequency of sucking during the 1st 10 min of posttreatment; $\pm$ figures refer to standard errors of the mean.)

20 sec/min was due almost entirely to a highly consistent decrease in rhythmic sucking [$F(1,18) = 31.01$, $p < .01$]; ANOVA performed on arrhythmic sucking data alone revealed no differences over time. Pups that had received milk during treatment engaged in significantly more sucking during the 30-min posttreatment phase than pups which received no milk during the treatment phase [$F(1,18) = 12.83$, $p < .01$]. Again, this difference was attributable to differences in rhythmic sucking [$F(1,18) = 13.01$, $p < .01$].

Two observations may be made about these data. First, frequency of rhythmic sucking declines with continued suckling in the absence of milk, a phenomenon we have observed previously (Brake & Hofer, 1980; Brake et al., 1979). Second, the effects of milk delivery on rhythmic sucking do not diminish immediately upon cessation of milk delivery, but persist for some time thereafter.

Experiment II

Since milk delivery affects the frequency of the pup's sucking on the nipple, it is possible that the patterning or duration of milk deliveries might affect the pup's sucking and nipple attachment behaviors from feeding to feeding, or even from day to day. In this experiment, we began to test this hypothesis. If the hypothesis is valid, then we might expect to see changes in the pup's sucking behavior from 1 day to the next as a consequence of how it was fed milk on the 1st day; if our hypothesis is not valid, then experience with different milk delivery schedules on the 1st day should not differentially affect sucking on the next day. This preliminary test of our hypothesis was severe in the sense that we were asking whether schedule-induced differences might be retained from 1 day to the next, rather than from 1 feeding to the next, but conservative in the sense that we utilized milk-delivery schedules that were extremely varied and not simply minor variations of the dam's naturally occurring pattern of milk letdown.

Method

Subjects

The subjects were 30 male and 30 female 11–13-day-old Wistar rat pups. Pups were culled and housed as described in Experiment I.

Design

Six groups of 11–13-day-old pups were run, each consisting of 5 males and 5 females drawn from an average of 6 different litters. On Day 1, maternally deprived pups in 5 groups received 2 hr of suckling experience while jaw-muscle EMG patterns were recorded as in the previous experiment. Each group received milk (half-and-half) or water, according to a different delivery schedule. The schedules were deliberately selected to be extremely varied, although pups eventually received the same total volume of fluid. Pups in the 6th group received no milk delivery or suckling experience on Day 1. On experimental Day 2, after 24 hr of isolation, pups in all 6 groups were recorded during 1 hr of suckling experience, this time in the absence of any milk or water delivery so that effects of Day 1 schedules might be observed unobscured by the immediate effects of milk delivery.

Procedure

Day 1. Twenty-four hours prior to treatment on Day 1 all pups were separated and operated on as described in Experiment I. Body weights and rectal temperature were recorded immediately prior to treatment. All pups, except those in the nonsuckling group (see below), were then allowed to suckle an anesthetized, nonlactating dam, 1 at a time, for 2 hr; EMG was recorded as in Experiment I.

Pups in the group designated Continuous Milk (CM) received milk through the tongue cannula from an open-end reservoir system throughout the 2-hr recording session. The free end of the PE 10 tongue cannula was attached to a 30-gauge needle which was then attached to a 1-ml syringe. The plunger was removed from the syringe and the syringe was filled with milk and vertically suspended 20 cm above the suckling pup. We knew from pilot work that pups would receive about .01 ml/min of milk through siphoning action alone and could greatly increase this rate of flow (up to .02–.03 ml/min) by sucking vigorously. Thus, milk was freely available during the 2 hr, but the rate of delivery was contingent largely upon the pup's behavior. These pups received an average of 1.80 ml during the 2 hr.

Pups in the group designated Pulsed Milk (PM) received a .20–.25-ml pulse of milk via the tongue cannula every 15–20 min. Milk delivery was not response-contingent. This schedule resembles the dam's natural pattern of milk letdown (Lincoln, Hill, & Wakerley, 1973). These pups received the same total volume of milk (1.80 ml) during the 2-hr session as pups which received milk continuously.

Pups in the group designated No Milk/Continuous Milk Off (NM/CMO) received no milk during the 2-hr suckling session, but instead received milk 20–30 min following the suckling session while isolated in warm, moist plastic tubs. Milk was infused via the tongue cannula over a 2-hr period so that these pups eventually received the same amount of milk (1.80 ml) as did pups which received milk continuously.

Pups in the group designated Continuous Water (CW) were treated similarly to pups which received milk continuously (CM) except that they received tapwater as they suckled. These pups received an average of 2.86 ml over the 2 hr, exactly the amount of fluid that they would have received through the siphoning action alone; sucking apparently had no effect on the rate of water delivery.

Pups in the group designated No Milk (NM) were allowed to suckle the anesthetized dam for 2 hr without receiving any milk. Pups in the group No Suckling (NS) were not allowed to suckle the dam at all. Pups in both of these groups, however, received an average of 1.80 ml of milk by gavage (this occurred 30 min following suckling of NM pups).

Following treatment (or gavage), pups were placed in small plastic tubs and returned to the incubator, where they remained for 24 hr.

Day 2. All pups were allowed to suckle an anesthetized dam for 1 hr. EMG was continuously recorded. No pup in any group received milk (or water) during the suckling session.

Results and Discussion

Day 1

Body weights and temperature. Pups in all 7 groups lost an average of 1.56 g during the first 24 hr of deprivation, from a mean weight of 28.2 g at the time of separation from the mother to a mean weight of 26.7 g prior to Day 1 treatment. Weight losses were essentially the same in all groups. Body temperatures were maintained throughout the deprivation period at an average 34.5°C.

Sucking. Figure 3 presents the Day 1 sucking data (rhythmic and arrhythmic combined) as well as data from a group of pups deprived for only 1 hr and then allowed to suckle an anesthetized dam in the absence of milk (designated No Deprivation Control; adapted from Brake et al., 1982 and Brake et al., 1979), and from a group of pups suckling in a normal mother-litter situation (designated Normal Litter Sucking; adapted from Shair et al., 1982). These data will be referred to in the discussion.

Fig. 3. Mean frequency of sucking (sec/min of time attached to the nipple) on Day 1 ($\pm$ figures refer to standard errors of the mean). "No Deprivation" and "Normal Litter" groups in right panel adapted from other sources (see text).

Most pups spent at least half the time they were attached to the dam's nipples engaged in some form of sucking. Even so, pups that received milk continuously (CM) sucked more frequently than did pups that received pulses of milk (PM) [ANOVA, $F(4,45) = 9.80$, $p < .01$; post hoc test, Scheffé $F(1,45) = 5.10$, $p < .01$], and PM pups sucked more frequently than did pups in the other 3 groups [Scheffé $F(3,45) = 3.34$, $p < .05$]. As in Experiment I, these changes in overall sucking (rhythmic and arrhythmic sucking combined) can be attributed to changes in rhythmic sucking (Table 1). Thus, CM pups engaged in more rhythmic sucking than did pups in all other groups [Scheffé $F(4,45) = 2.69$, $p < .05$]. This pattern of results suggests that milk delivery activates or excites 11-13-day-old pups, and that increased rhythmic sucking may simply be a specific behavioral indicant of such activation. However, in light of data suggesting that pups are capable of associative learning while suckling (Brake, 1981), and data suggesting that rhythmic sucking leads to increased milk consumption (Brake et al., 1982), it is also possible that pups receiving milk continuously might suck most frequently because they have learned that increased rhythmic sucking results in the delivery of more milk. The same cannot be said of pups receiving water continuously. They engaged in far less sucking than pups in either of the milk-delivery groups, consuming no more water than required by the siphoning action of the delivery system. This suggests that water may be a relatively unpalatable substance or that its relatively rapid rate of delivery may itself be aversive.

Another factor which served to increase sucking rates was maternal deprivation. All of the pups were deprived prior to the test sessions and sucking rates were uniformly higher in all groups than in nondeprived pups from our previous studies (right panel, Fig. 3). Even those experimental pups that received no milk and that engaged in the least sucking (NM pups, for example) appear to suck as frequently as normally mothered pups that receive a pulse of milk every 5-10 min.

Nipple attachment. Pups that received milk continuously (CM) spent less time off the nipple than pups in all other groups [Table 1; ANOVA, $F(4,45) = 16.39$, $p < .01$; post hoc test, Scheffé $F(1,45) = 4.12$, $p < .05$], while pups that received water continuously

TABLE 1. *Rhythmic Sucking, Arrhythmic Sucking, and Time Spent Unattached to a Nipple on Day 1, Experiment II.*

Group	Day 1		
	Rhythmic Sucking ($\bar{x}$ sec/min attached)	Arrhythmic Sucking ($\bar{x}$ sec/min attached)	Time Unattached to Nipple (%)
Continuous Milk (CM)	31.7*	15.2	0.5*
Pulsed Milk (PM)	19.8	18.2	9.7
Continuous Water (CW)	10.0	18.2	37.6*
No Milk/Continuous Milk Off (NM/CMO)	10.1	14.8	6.8
No Milk (NM)	9.8	15.3	9.7
No Suckling (NS)	—	—	—

*Indicates their values differ statistically from others in column.

(CW) spent more time off the nipple than did pups in all other groups [Scheffé $F(1,45) = 15.48, p < .01$]. These data seem to reflect the quality of the suckling experience as accurately as do the sucking data. Pups that received milk continuously almost never left the nipple, while pups that received pulses of milk while sucking, or no milk at all, spent 10% of the session off the nipple, and pups that received water spent 37% of the time unattached to a nipple. It might be argued that if milk delivery is reinforcing, then all pups that received milk should have remained on the nipple. But CM pups were provided milk continuously while PM pups experienced milk intermittently; this latter sequence may even be a cue for leaving the nipple and reattaching to another one (Cramer, Blass, & Hall, 1980).

Day 2

Weights and temperatures. Pups lost an average of 1.36 g during the previous 24-hr deprivation to bring their mean weights down to 25.6 g prior to testing. There were no significant differences among groups in weight loss. Rectal temperatures continued to be maintained at an average of 34.5°C for all groups.

Nipple attachment. Pups that had received water on Day 1 (CW) spent considerably less test time off the nipple on Day 2 than on Day 1, while pups that had received milk continuously on Day 1 (CM) spent relatively more time off the nipple on Day 2 than on Day 1 (Table 2). The net result was that pups of all groups did not differ in the percentage of time spent off the nipple on Day 2.

Sucking. Pups that received milk continuously on Day 1 (CM) engaged in the most sucking during the Day 2 session [Fig. 4; ANOVA, $F(5,54) = 5.32, p < .01$; Scheffé $F(1,54) = 5.68, p < .01$]. Pups that had received fluids via the intraoral cannula on Day 1 (whether milk or water, whether delivered continuously or in pulses, whether or not delivered while the pup was attached to the nipple), engaged in more sucking than pups that had received no intraoral milk (NM) or no suckling experience (NS) [Scheffé $F(4,54) = 2.45, p < .05$]. And, as on Day 1, group differences in overall sucking can be attributed to differences in rhythmic sucking (Table 2). Thus, the pups that had received no milk or

TABLE 2. *Rhythmic Sucking, Arrhythmic Sucking, and Time Spent Unattached to a Nipple on Day 2, Experiment II.*

Group	Day 2		
	Rhythmic Sucking ($\bar{x}$ sec/min attached)	Arrhythmic Sucking ($\bar{x}$ sec/min attached)	Time Unattached to a Nipple (%)
Continuous Milk (CM)	33.4	19.4	18.3
Pulsed Milk (PM)	24.3	20.2	12.3
Continuous Water (CW)	22.5	20.2	15.3
No Milk/Continuous Milk Off (NM/CMO)	22.2	16.7	11.6
No Milk (NM)	12.4*	16.4	14.0
No Suckling (NS)	14.5*	14.9	4.6

*Indicates these values differ statistically from others in column.

Fig. 4. Mean frequency of dry sucking (sec/min of time attached to the nipple) on Day 2 ($\pm$ figures refer to standard error of the mean). "No deprivation" and "Normal Litter" groups in right panel adapted from other sources (see text).

suckling experience on Day 1 (NM, NS) engaged in less rhythmic sucking than pups in all of the other groups [Scheffé $F(5,54) = 2.42, p < .05$].

These findings suggest that a single 2-hr experience with a particular milk delivery schedule can differentially influence the frequency of a pup's sucking 24 hr later and in the absence of any further milk delivery. This is important because it reveals an unexpected plasticity in the control of sucking patterns in pups with nearly 2 weeks suckling experience, and because the effects of the differential feeding experience were retained for so long in such young animals.

Experiment III

The results of the previous experiments, as well as those of others (Brake, 1981; Brake et al., 1982; Letz et al., 1978), demonstrate that rat pups as young as 11 days of age perceive milk delivery as a salient event and are capable of discriminating suckling associated with milk delivery from nonnutritive suckling. Rat pups apparently become even more responsive to the nutritive contingencies associated with feeding between 15 and 25 days of age (Kenny et al., 1979; Williams, Hall, & Rosenblatt, 1980), which is the beginning of the weaning period. In this experiment we wished to determine whether experience with different milk delivery contingencies over the several days of the normal weaning period might differentially affect sucking, and perhaps even contribute to the weaning process itself. Specifically, we asked (1) whether repeated exposure to continuous response-contingent milk delivery, or continuous nonnutritive suckling, from postpartum Days 14 to 18, differentially affects the pups' behavior during a suckling test at 19 days of age, and (2) whether 5 days' experience with these schedules affects sucking more substantially than a single day's experience.

Method

Subjects

The subjects were 32 male and 32 female Wistar rat pups.

Design and Procedure

Eight groups of pups were run, each consisting of 4 males and 4 females drawn from an average of 6 different litters. All pups were outfitted with EMG recording electrodes and tongue cannula as described in Experiment I.

Three groups of pups were separated from the dam at 13 days of age and housed in groups of 3 for the duration of the experiment. These pups were kept warm by placing the housing terraria in an incubator. Pups were provided with wet mash, milk formula (Esbilac), and water ad libitum. Beginning with the next day (14 days postpartum), and continuing for the next 4 days, 2 of these 3 groups were allowed to suckle an anesthetized dam for 2 hr each day. Pups in 1 group (CM-14-18) were allowed to consume milk continuously in the manner previously described, while pups in the 2nd group (NM-14-18) received no milk while suckling. Pups in the 3rd group (NS-14-18) were not allowed to suckle the dam from 14 to 18 days postpartum. CM-14-18 pups consumed approximately 1.90 ml of milk during each day's session and pups in the other 2 groups received this volume of milk by gavage each day. At 19 days postpartum, pups in all 3 groups were allowed to suckle an anesthetized dam for 60 min. As in Experiment II, pups received no milk during the suckling "test" so that any effects of past experience might be observed unobscured by the immediate effects of milk delivery. Jaw-muscle EMG was recorded by polygraph during the 60-min test. The amount of time pups spent unattached to a nipple was also noted.

Another 3 groups of pups were separated from the dam at 17 days postpartum and placed in warm terraria in groups of 3. The next day (18 days postpartum), pups in 1 of each of these groups received the same kind of suckling experience as did pups in either group CM-14-18, NM-14-18, or NS-14-18. One group of pups was allowed to suckle an anesthetized dam for 2 hr while receiving milk continuously (CM-18), 1 group of pups suckled the dam for 2 hr but received no milk (NM-18), and 1 group of pups was not allowed to suckle the dam during the day (NS-18). CM-18 pups consumed an average of 2.08 ml of milk during the 2 hr and pups in the other 2 groups received this volume of milk by gavage. Thus, these 3 groups of pups were treated similarly to the 1st 3 groups of pups, except that they received differential suckling experience for only 1 day. As mentioned above, at 19 days postpartum, all pups were allowed to suckle an anesthetized dam for 60 min. Pups received no milk during this suckling test. Jaw-muscle EMG and the amount of time spent unattached to a nipple were recorded.

Pups in the final 2 groups were not separated from the dam until 24 hr or 4 hr prior to the suckling test at 19 days of age. Pups in these groups, as with pups in the other 6 groups, received no milk during the test.

Results and Discussion

Body Weights

Pups that received only 2 hr of suckling experience per day from 14 to 18 days of age (CM-14-18, NM-14-18) or no suckling experience during this time (NS-14-18) showed little weight change during the 5 days. Normally reared pups (those separated 24 hr or 4 hr prior to testing) showed substantial weight gain over this same period. The average weight at 13 days of age for pups in groups CM-14-18, NM-14-18, and NS-14-18 was 31.8 g. The average weight at 19 days of age was 34.4 g. Normally reared pups weighed 31.0 g at 13 days of age and 36.0 g at 19 days of age (following 24 hr of maternal deprivation).

Sucking

Pups that had received milk continuously during the 2-hr sucking sessions engaged in more sucking during the nonnutritive suckling "test" than did pups in any of the other groups. This was true of both CM-14-18 pups, which had received 5 days' treatment [Fig. 5, left panel; $F(2,27) = 4.92, p < .01$], and of CM-18 pups, which had received only 1 day of treatment [Fig. 5, right panel; $F(4,45) = 6.92, p < .01$]. Thus, varied feeding experience differentially affected sucking behavior at weaning, just as they did in younger pups. Contrary to our expectations, however, pups allowed to consume milk during five 2-hr suckling sessions (CM-14-18) did not suck more frequently than pups allowed only one 2-hr session (CM-18). Similarly, pups which received no milk during the five 2-hr session (NM-14-18) did not suck less frequently than pups allowed only 1 "dry" suckling session prior to the test session (NM-18).

All of the pups in this experiment engaged in less sucking during the suckling test than did the younger pups of the previous experiments (Fig. 6). This was true even of those pups which had received milk continuously during treatment [Scheffé $F(2,54) = 11.66, p < .01$].

Nipple Attachment

Pups deprived from 13 to 18 days of age (NS-14-18) spent significantly more time unattached to a nipple at 19 days of age than did normally mothered pups (Table 3). Repeated experience with nonnutritive sucking (NM-14-18) did nothing to modify this decline in nipple attachment. However, the addition of milk delivery to these brief experiences at the maternal ventrum caused CM-14-18 pups to remain attached to the nipple as much of the time as normally reared pups during the test at 19 days of age, and for much longer than either NS-14-18 pups or NM-14-18 pups [$F(2,27) = 3.21, p < .05$]. No such effect was generated by the single 2-hr experience with milk on the day prior to the final Day 19 reading. Thus, while early and complete separation of the pups from their mothers for 5 days prior to testing did not result in any reduction in the frequency of sucking when reunited, the overall amount of time spent sucking was significantly lessened by the reduction in time these pups spent attached to the nipple. In this way, the process of weaning was taking place more rapidly in these early-separated

Fig. 5. Mean frequency of sucking (sec/min of time attached to the nipple) at 19 days of age for pups receiving treatment from 14-18 days of age (left panel) and at 18 days only (right panel; $\pm$ figures refer to standard errors of the mean).

Fig. 6. Mean frequency of rhythmic sucking (sec/min of the time attached to the nipple) on test day for pups treated at 12, 18, and 14-18 days of age ($\pm$ figures refer to standard errors of the mean).

animals. The results of this experiment show that this weaning process was largely attributable to the absence of milk delivery and not simply to the absence of the opportunity to suck on the maternal ventrum over that period of time. Pups that received no milk during daily sucking experience were like totally separated pups, spending 25% of the time off the nipple, whereas those fed continuous milk during the same daily experiences with their mothers were virtually identical to normally mothered pups in their nipple attachment times (10% of the time off the nipple). It has been reported that newborn pups separated from the mother from 3 to 5 days of age (and fed intragastrically) lose the ability to suckle unless provided with brief periodic opportunities to locate and attach to a nonlactating nipple (Stolhoff, Kenny, Blass, & Hall, 1980). The current data suggest that older pups must also be provided with milk in order to maintain age-appropriate interest in the nipple, and that in the absence of milk delivery, some aspects of weaning are accelerated.

General Discussion

The results of these experiments demonstrate that feeding experiences on 1 day can affect the frequency of the pup's sucking and the tenacity with which it attaches to the nipple on subsequent days. These effects were most apparent in pups given experience

TABLE 3. Percent of Time Spent Unattached to a Nipple During the Suckling Test at 19 Days of Age.

Group	Test Day (%)	
	Treatment 14-18	Treatment 18
Continuous Milk (CM)	9.8*	15.5
No Milk (NM)	25.1	3.6
No Suckling (NS)	23.9	7.3
24-hr Deprivation	—	9.2
4-hr Deprivation	—	8.5

*Indicates this value differs statistically from others in column.

with continuous milk delivery. They engaged in more rhythmic sucking, both immediately and 24 hr later, than pups in any of the other groups. Continuous milk delivery also reduced the amount of time pups spent away from the nipple, and pups which received consecutive experiences with continuous milk delivery over a period of 5 days were significantly less susceptible than controls to the effects of early maternal separation, as reflected by the very small percentage of time they spent unattached to a nipple. In a sense, experience with continuous milk delivery delayed the onset of weaning.

The particularly high frequency of sucking and nipple attachment observed in pups which had received milk continuously seems to be the result of at least 3 factors: (1) fluid was experienced continuously during treatment; (2) fluid was experienced contingent on sucking; and (3) the fluid was milk, not water. There are several ways in which these factors might affect subsequent sucking. Pups receiving milk during treatment might become so much more sensitized to perioral stimulation than pups receiving no milk that even contact with a dry nipple 24 hr later evokes excessive sucking. A tentative precedent for this explanation can be found in the demonstration that pups 10–15 days of age display an unexpected form of response sensitization (leg flexion) to repeated presentations of a stimulus (electric shock) that in adult animals leads to response habituation (Stehouwer & Campbell, 1978). An alternative explanation is that pups may have learned to suck more frequently as a consequence of exposure to what could be considered continuous milk reinforcement. We have demonstrated elsewhere that pups receiving milk in this fashion are capable of associating the milk they receive with novel cues in the environment (Brake, 1981). In any case, the data suggest that the dam's naturally occurring intermittent milk delivery schedule (e.g., Lincoln et al., 1973) elicits more frequent sucking than would result if the dam were dry (e.g., Shair et al., 1982), but not as much sucking as if milk delivery were continuous.

Finally, the mode of sucking most affected by the different milk delivery schedules is rhythmic sucking. This sucking pattern resembles the nutritive pattern of sucking displayed by human infants (Wolff, 1968), and according to other work (Brake et al., 1982) and our ongoing research, is apparently an important component of the rat pups' ingestive behavior. A clear implication of these findings is that highly successful feeding experiences might subsequently lead to increased sucking and ingestion of more milk. Several successful feeding experiences might even slow the pup's transition from suckling to the consumption of solid food. Although a number of factors play a role in weaning, such as the mother's behavior towards the pups (Bolles & Woods, 1964), and the maturation of neural systems in the pup (Williams, Rosenblatt, & Hall, 1979), the present experiments suggest that the manner in which the pup has been accustomed to receiving milk from the dam might also play a role in weaning.

Notes

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References

- Bolles, R. C., and Woods, P. C. (1964). The ontogeny of behavior in the albino rat. *Anim. Behav.*, 12: 427–441.
- Brake, S. C. (1981). Suckling infant rats learn a preference for a novel olfactory stimulus paired with milk delivery. *Science*, 211:506–508.

- Brake, S. C., and Hofer, M. A. (1980). Maternal deprivation and prolonged suckling in the absence of milk alter the frequency and intensity of sucking responses in neonatal rat pups. *Physiol. Behav.*, 24:185-189.
- Brake, S. C., Sager, D. J., Sullivan, R., and Hofer, M. A. (1982). The role of intraoral and gastrointestinal cues in the control of sucking and milk consumption in rat pups. *Dev. Psychobiol.*, 15:529-541.
- Brake, S. C., Wolfson, V., and Hofer, M. A. (1979). Electrophysiological patterns associated with non-nutritive sucking in 11-13-day-old rat pups. *J. Comp. Physiol. Psychol.*, 93:760-770.
- Cramer, C. P., Blass, E. M., and Hall, W. G. (1980). The ontogeny of nipple-shifting behavior in albino rats: Mechanisms of control and possible significance. *Dev. Psychobiol.*, 13:165-180.
- Hall, W. G., and Rosenblatt, J. S. (1977). Sucking behavior and intake control in the developing rat pup. *J. Comp. Physiol. Psychol.*, 91:1232-1247.
- Hall, W. G., and Rosenblatt, J. S. (1978). Development of nutritional control of food intake in suckling rat pups. *Behav. Biol.*, 24:413-427.
- Kenny, J. T., Stolhoff, M. L., Bruno, J. P., and Blass, E. M. (1979). Ontogeny of preference for nutritive over non-nutritive suckling in albino rats. *J. Comp. Physiol. Psychol.*, 93:752-759.
- Letz, R., Burdette, D. R., Gregg, B., Kittrell, M. E., and Amsel, A. (1978). Evidence for a transitional period for the development of persistence in infant rats. *J. Comp. Physiol. Psychol.*, 92:856-866.
- Lincoln, D. W., Hill, A., and Wakerley, J. B. (1973). The milk-ejection reflex of the rat: An intermittent function not abolished by surgical levels of anesthesia. *J. Endocrin.*, 57:459-476.
- Shair, H., Brake, S. C., and Hofer, M. A. (1982). Suckling in the rat: Evidence for patterned behavior during sleep. Unpublished manuscript.
- Stehouwer, D. J., and Campell, B. A. (1978). Habituation of the forelimb-withdrawal response in neonatal rats. *J. Exp. Psychol: Anim. Behav. Proc.*, 4:104-119.
- Stolhoff, M. L., Kenny, J. T., Blass, E. M., and Hall, W. G. (1980). The role of experience in suckling maintenance in albino rats. *J. Comp. Physiol. Psychol.*, 94:847-856.
- Williams, C. L., Hall, W. G., and Rosenblatt, J. (1980). Changing oral cues in suckling of weanling-age rats. *J. Comp. Physiol. Psychol.*, 94:472-483.
- Williams, C. L., Rosenblatt, J. S., and Hall, W. G. (1979). Inhibition of suckling in weanling-age rats: A possible serotonergic mechanism. *J. Comp. Physiol. Psychol.*, 93:414-429.
- Wolff, P. H. (1968). Suckling patterns of infant mammals. *Brain Behav. Evol.*, 1:354-367.