

Postsuckling Behavioral Arousal in Weanling Rats (*Rattus norvegicus*)

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Twenty-day-old litters and their dams were observed in seminatural habitats consisting of a nest compartment and adjacent open field that contained powdered rat chow. It was found that pups displayed marked bursts of activity after suckling. Independent feeding reliably followed nursing bout termination (Experiment 1). Nipple withdrawal, with or without milk transfer, induced behavioral arousal whereas withdrawal of thermotactile and conspecific odor cues did not (Experiments 2–3). Increased thermogenesis was observed following milk transfer (Experiment 4). Finally, preweanling pups (10- to 12-day-olds) also displayed postsuckling arousal within the confines of the nest; full locomotor expression of this arousal was not evident until weaning age (Experiment 5). It was concluded that postsuckling arousal in weanlings functions to stimulate activities performed away from the nest and suckling, propelling pups into the field where feeding begins.

Weaning, in a strict sense, refers to the ontogenetic shift of ingestive mode, from suckling exclusively the mother's milk to independent feeding on solid foods. Weaning in domesticated Norway rat pups (*Rattus norvegicus*) is a dramatic but rather gradual affair. The young reliably begin to ingest solid food around Day 18 (Thiels, Alberts, & Cramer, 1990). Time spent suckling begins to diminish measurably on about Day 20 (Cramer, Thiels, & Alberts, 1990), and time spent consuming solid food increases (Thiels et al., 1990). By Day 34, the weaning process is usually complete, and pups no longer suckle.

An understanding of weaning may require looking beyond the mechanisms traditionally viewed as those controlling ingestive behavior. It has long been recognized that juvenile rats present a distinct picture of behavioral propensities around the time of weaning onset. Small (1899) described the weanling-aged pups as "inordinately curious," reflecting perhaps the bursts of boisterous "hyper-activity" noted by Bolles and Woods (1964). In subsequent studies, Campbell and his associates systematically quantified the ontogeny of "behavioral arousal" in rats. They used a variety of devices, including stabilimeters and video recordings, to derive general and specific kinds of measurements of behavioral activities. These studies established that both

locomotor activity as well as specific behaviors tend to increase around Day 15 and may continue to become more frequent through weaning (Campbell, Lytle, & Fibiger, 1969; Campbell & Mabry, 1972; Moorcroft, Lytle, & Campbell, 1971; Randall & Campbell, 1976; see also Bolles & Woods, 1964). Different age-related patterns of activity occurred in different contexts. For instance, individually tested rat pups show a "peak" in activity on Day 15 (Campbell et al., 1969). When pups are tested among their littermates and with the mother, however, behavioral activity increases monotonically from Days 15 to 30 (Randall & Campbell, 1976). Thus, although contextual factors, such as social stimuli, environment size, and configuration can affect the various activity scores, most measures reveal age-related changes in activity—both general and specific—just prior to and during the weaning phase.

More recently, Thiels et al. (1990) described, both qualitatively and quantitatively, pup activity from Days 14–34. They used continuous, time-lapse video observations to measure daily the durations and frequencies with which pups engaged in suckling, independent feeding and drinking, play-fighting, self-grooming, and sleep. Litters were observed in habitats where food was in a separate location from the nest, thus approximating natural conditions (Calhoun, 1962; Von Steiniger, 1950). Thiels et al. (1990) noted three sequential stages in the pups' behavioral profiles. During the first phase, from Days 14–17, pups showed a stable pattern of behavior consisting primarily of sleeping and nursing. The second phase (Days 18–25), in contrast, was characterized as a time of "general behavioral eruption" (p. 507), during which activities such as feeding, drinking, play-fighting, and self-grooming emerged or increased markedly. Many of these behaviors were primarily observed outside of the nest and independent of the dam. The sequence of these behaviors in relation to rest and sleeping were less stable than during previous and subsequent phases. In the third phase, encompassing Days 25–34, pups' behavior stabilized. Novel behaviors no longer emerged and the frequency of specific behaviors was stable across days. In the present study we explore the possibility that in-

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creased behavioral arousal may facilitate the weaning transition by moving pups away from the nest and into an area that contains solid food. In particular, we focus on behavioral arousal following suckling and the relation of this arousal to independent feeding. In Experiment 1, we conducted a sequential analysis of the events that preceded the independent feeding bouts of weanling-aged pups. We supplemented this quantitative analysis with qualitative descriptions of the young's behavior. Experiments 2A–C and 3 were used to examine separate elements of the nursing interaction to isolate and identify the cues that stimulate the pups' behavioral arousal. In Experiment 4, we examined the possibility that thermogenesis is potentiated after suckling, which might facilitate nest egression and subsequent ingestion of solid food. Finally, in Experiment 5, we determined whether preweaning pups display suckling-induced behavioral arousal.

Experiment 1: Relation Between Suckling and Independent Feeding Bouts

Experiment 1 provided observational data on the events that reliably precede episodes of independent feeding by rat pups living with their dam in a seminatural habitat. Pilot observations suggested that pups displayed bursts of behavioral activation following termination of a nursing bout; in this aroused state they seemed especially likely to leave the nest and to ingest solid food. We therefore examined in this experiment the relation between termination of a suckling bout and the onset of independent feeding. We used 20-day-old pups because they are a few days past the first ingestion of solid food (Day 18; Thiels et al., 1990), but still well within the early phase of the weaning transition (Cramer et al., 1990). We analyzed from twelve 1-hr-long videotaped sessions sequential events following suckling to determine whether there was a close relation between termination of a suckling bout and onset of independent feeding. The goal of Experiment 1 was to assess the likelihood that suckling and independent feeding bouts are more closely related in time than are other events or than by simple chance occurrence.

Method

Subjects. Subjects were 14 Sprague-Dawley dams and their 20-day-old litters. The rats were bred and born in the Animal Behavior Laboratory colony at Indiana University. The original stock was obtained from Charles River Labs, Inc. (Wilmington, MA). Cages were checked daily around 1,700 hr for births (day of birth = Day 0). Litters were culled to 8 pups (4 males and 4 females) on Day 3. All pups were housed with their mother and littermates in standard maternity cages (47 cm long \times 26 cm wide \times 21 cm high) and were maintained on a 16:8-hr light–dark cycle. Ambient temperature was between 21°C and 23°C.

Seminatural habitat. The seminatural habitat consisted of a field (33.5 cm long \times 33 cm wide \times 12.5 cm high) connected by a 19.0 \times 7.5 \times 6.5 cm tunnel to a separate nest area (47 \times 26 \times 21 cm; see Gerrish & Alberts, 1996, Figure 1). Powdered chow was available in two glass dishes (11.0 cm diameter \times 5.0 cm high) each filled with an abundance of chow (60.0 g), and located in the field. This arrangement was used to simulate natural con-

ditions in which food is typically stored in an area of the burrow separate from the nest (Calhoun, 1962; Von Steiniger, 1950). Therefore, in the present study, pups had to leave the nest to encounter solid food. Water was supplied ad libitum in the nest. The animals mainly resided and nursed in the smaller nest area and made excursions to the field.

The design of the Gerrish and Alberts (1996) study required that the ambient temperature in the field be either warm (30°C; $n = 5$ groups), moderate (21°C; $n = 5$ groups), or cold (10°C; $n = 4$ groups). In brief, the field was designed to regulate and maintain stable temperatures. The field was surrounded by a copper tube from which radiated aluminum fins; this "slant fin tubing" was connected to a temperature-controlled water circulator that enabled environmental temperature control. The field was insulated on the sides and bottom with styrofoam 5.1 cm thick, and the top was covered with a double layer of Plexiglas lids to allow full view of the animals and assist the insulation. The nest area was maintained at room temperature (approximately 21°C) and consisted of a standard polypropylene maternity tub, within which was a small hardware cloth nestbox 19.0 \times 13.0 \times 8.0 cm. For a detailed description of this habitat, refer to Gerrish and Alberts (1996). In that study we observed dams and their litters within this seminatural habitat from Days 14 to 22 to determine whether the timing of first nest egression and first ingestion of solid food was influenced by environmental temperature. Results indicated that the warmer the field temperature, the earlier pups egressed from the nest and the earlier they began to ingest solid food. Thus, to leave the nest and contact solid food, weanlings must encounter an appropriate thermal milieu. In the present study, we observed pups that were already reliably leaving the nest and ingesting solid food; possible interactions of field temperatures and behavior are discussed later.

Procedure. Dams were gradually acclimated to a dual-compartment, seminatural habitat used for this experiment. The acclimation procedure began on postpartum Days 6–8, when the mother and litter were placed in an apparatus consisting of two maternity tubs connected by a tunnel. One maternity tub, designated the *nest area*, contained soiled bedding and was darkened by a cover; the attached maternity cage, designated the *food area*, contained food pellets and a water bottle and was uncovered. Midway in the connecting tunnel (19.0 \times 7.5 \times 6.5 cm) was a swinging door (5.0 cm high \times 6.5 cm wide) that reduced heat exchange between the two compartments when experimental conditions created a gradient across the two areas. Preliminary observations indicated that this acclimation period was especially useful for the rats' familiarization with the swinging door. On postpartum Days 11–12 the nest and tunnel of the acclimation cage were detached from the food area and attached to the field.

On Day 11 or 12, the litter was weighed and two pups of average body-weight (one male and one female) were chosen as focal pups and were marked with Clairol black hair dye for identification. On Day 20, the study began.

The animals' behavior was recorded with time-lapse video recorder (Gyrr Model TLC1400, Anaheim, CA; 12:1 record: playback ratio) and two cameras (Panasonic WV-BL90); one camera was directed on the field and the other on the nest. A screen splitter was used to view both areas of the habitat simultaneously. We recorded from 2130 hr to 0930 hr, that is primarily during lights off (2300 hr to 0700 hr), because the dam is more active during the dark phase and is known to influence the pups' early feedings (Galef, 1971). Red lights (25 watts) enabled viewing of the animals during the dark phase. Pilot studies indicated that these 12 hr of observation provided information equivalent to that collected with 24 hr of daily observations.

During playback, an observer used a keyboard-activated com-

puter program to quantify specific events defined as follows: (a) *suckling* was the duration that the focal pups spent attached to the nipples of the dam (Brief detachment from the nipple after milk letdowns, i.e., nipple shifts, were included in the total duration of time spent suckling.), (b) *independent feeding* was the duration that each focal pup spent in oral contact with food, (c) *dam in field* was the duration that the dam spent in the field (More than half of a body length entering the tunnel was scored as in the field.), and (d) *pups in field* was the duration that each focal pup spent in the field (More than half of a body length entering the tunnel was considered entry into the field.).

Data analysis. The program used to tabulate the data provided a second-by-second account of each of the scored events, including the elapsed time during each period in which there was no activity. The program yielded a continuous record of the order in which the various behaviors occurred and the elapsed duration between behaviors.

To determine the relation between suckling and feeding, we applied the operational definitions mentioned earlier to performance levels for the behaviors. Data from each pair of focal pups within a litter (as stored within the computer file) were combined and treated as a single statistical observation. We adhered to this conservative approach, noting that pups within a litter share a common mother and, in the context of a study of ingestive behavior, pups sharing a nursing mother are less independent than pups with different dams. Nursing with milk transfer usually involves simultaneous suckling by most of the pups in a litter, so we adapted this criterion to the focal pups' suckling and their feeding, that is, that they both display the behavior.

Suckling was noted when both focal pups were attached to the nipples of a quiescent dam and the bout was scored as terminated when both focal pups were detached. This criterion helped identify suckling bouts during which milk transfer was likely to occur (namely, when more than 4 pups suckled for longer than 10 min; see Lincoln, Hill, & Wakerley, 1973) and avoided inclusion of instances in which one or two pups might briefly grasp a nipple. *Independent feeding* was noted when both focal pups spent a total of at least 100 s per 45-min bin at a food bowl, in oral contact with food. This criterion ensured that pups were more likely consuming solid food rather than merely sampling small quantities. We applied the same stringent criteria of dual-expression to the focal pups: Both focal pups were required to feed during a temporal bin, but they did not have to ingest the food simultaneously.

From these data, we sought to determine the temporal relation

between termination of a suckling bout and onset of independent feeding bouts. Pilot observations from videotaped sequences indicated that pups typically began to ingest solid food within about 10 min following termination of a suckling bout. However, the behavioral arousal following suckling was observed for roughly 45 min past termination of the suckling bout. Feeding was intermittent between other activities during this 45-min period. Based on these observations, the 12-hr activity period for each litter was divided into bins that fit into a 2×2 contingency table as follows: A new bin began at the completion of a suckling bout and lasted for 45 min following that bout, or a new bin began at the end of the 45-min period following a suckling bout and lasted until the end of the next suckling bout. Each bin was then assigned to one of four mutually exclusive categories (see Figure 1): (a) feeding occurred within 45 min of suckling, (b) no feeding occurred within 45 min of suckling, (c) feeding occurred that was not within 45 min of suckling, and (d) no feeding occurred within 45 min of a nonsuckling period. These frequency categories were collapsed across litters and a chi-square analysis was applied to the data.

Post hoc we performed two separate one-way chi-square analyses to test specific hypotheses about the nature of the relation between suckling and feeding. We compared all bins in which feeding took place to determine if feeding was more likely to occur within 45 min of suckling or not within 45 min of suckling, that is, independent of suckling. The null hypothesis was that either event was equally likely. Next, we compared all bins that were within 45 min of suckling to determine the likelihood of feeding. Thus, we asked whether suckling is more likely to lead to feeding than to not lead to feeding. The null hypothesis was that each event was equally likely.

Three circumstances disqualified bouts from inclusion in the data analysis. First, if the videotape ended before 45 min after a suckling bout, that bin was excluded. This criterion eliminated seven bins from the data set. Second, if one suckling bout led to another suckling bout, that is, suckling occurred within 45 min of a suckling bout, this bin was not included. This event was rare; it occurred only four times. Third, in some instances, the day's record began with suckling or suckling occurred within 12 s of the start of the tape. In such cases, the initial part of the tape was too short to include any events other than suckling and the initial portion of the tape was not included as a bin. Instead, the 45-min period following the suckling bout counted as Bin 1.

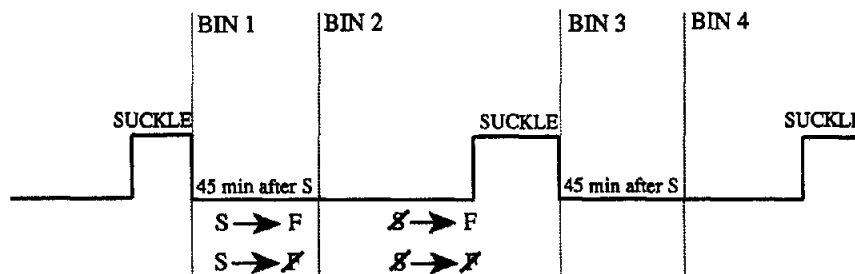


Figure 1. Schematic of bin divisions. New bins starting after termination of a suckling bout were either assigned to the category of feeding within 45 min of suckling or no feeding within 45 min of suckling (see Bin 1). New bins that began after this 45-min time period were either assigned to the category of feeding that was not within 45 min of suckling or no feeding occurred and it was not within 45 min of suckling (see Bin 2). Note that the length of Bins 2 and 4 could vary, as could the length of the suckling bout, whereas bins following termination of a suckling bout remained fixed at 45 min. Bins were given numbers arbitrarily to provide examples. S = suckling; F = feeding.

Results and Discussion

An overall chi-square analysis indicated that there was a relation between termination of a suckling bout and onset of independent feeding bouts (see Figure 2), $\chi^2(1, N = 124) = 23.35, p < .0001$. Among all the independent feeding bouts, 32 bouts occurred within 45 min of suckling, and only 10 were not within 45 min of suckling. We applied a one-way chi-square test to these data using expected frequencies of 21 (based on the null hypothesis that the events were equally likely). Results were significant, indicating that feeding was more likely to follow a suckling bout than to occur independently of a suckling bout, $\chi^2(1, N = 42) = 11.52, p = .0007$.

The data also indicated that of all bins that were within 45 min of suckling, 32 of those involved feeding and 25 did not. A one-way chi-square test was applied to the data using expected frequencies of 28.5 (based on the null hypothesis that either event was equally likely). Results indicated that both events were equally likely, $\chi^2(1, N = 57) = 0.86, p = .35$. Thus, suckling may or may not lead to feeding in rats just beginning to ingest solid food. However, when feeding occurs it is more likely to be after sucking than to be independent of suckling.

Recall that the subjects in the present experiment were also part of another study (Gerrish & Alberts, 1996) and encountered solid food in the field at one of three temperatures (see *Method* section). To assess whether field temperature affected the relation between suckling and feeding, we performed separate chi-square analyses for each group. There was a close relation between termination of a suckling bout and onset of an independent feeding bout at each temperature: warm, $\chi^2(1, N = 44) = 7.06, p < .01$; moderate, $\chi^2(1, N = 48) = 6.65, p < .01$; cold, $\chi^2(1, N = 32) = 10.86, p < .01$. We then performed separate one-way chi-square analyses to determine whether feeding was more likely to be preceded by suckling than to not be preceded by suckling. Even with so few litters ($n = 4$) tested, for pups in the cold condition feeding occurred more within 45 min of suckling than it did beyond 45 min of suckling: cold, $\chi^2(1,$

$N = 10) = 6.4, p = .01$. A similar trend, although not significant, was found for pups in the moderate and warm conditions: moderate, $\chi^2(1, N = 15) = 3.26, p = .07$; warm, $\chi^2(1, N = 17) = 2.9, p = .09$.

Our application of the chi-square test is rather novel and potentially complex: Bins were statistically independent but the same focal pups could appear in multiple bins. Nevertheless, we emphasize here the overall coherent pattern of the results. More important is that the present observations are described as the basis for a series of rigorously controlled experiments in which postsuckling activity is more clearly and cleanly defined. The present results are suggestive at least, and our basic interpretation was confirmed in the subsequent studies.

Observations of videotaped data suggested that pups displayed bursts of activity following suckling bouts that were not regularly seen at other times. This activity included considerable self-grooming, play-fighting, and running from nest to field; independent feeding reliably occurred after suckling bouts. There appeared to be no apparent order to these activities (recall, however, that pups had to egress from the nest to encounter solid food). Feeding was embedded within this 45 min postsuckling arousal; on some trips to the field pups would feed, whereas on other trips to the field they would not. Interestingly, pups appeared to act in unison with littermates rather than in synchrony with the dam. Consistent with the observations of Thiels et al. (1990), self-grooming, play-fighting, and feeding occurred in apparent independence of the dam's behavior. Specific behavioral components of arousal such as play-fighting and self-grooming seemed to increase the likelihood that pups would egress from the nest and encounter solid food. This idea is explored in Experiment 3, which involves quantification of the different behavioral components of arousal.

The present observations, in conjunction with the sequential data, suggest that either nursing or termination of a suckling bout may lead to a surge of activity associated with nest egression and subsequent exploration and ingestion of solid food. By virtue of triggering such bursts of activity that lead to behaviors in the field, including feeding, suckling might lead to its own demise.

	WITHIN 45 MIN. OF SUCKLING	NOT WITHIN 45 MIN. OF SUCKLING
FEED	Suckle → Feed 32 (19.31)	Suckle → Feed 10 (22.69)
NO FEEDING	Suckle → Feed 25 (37.69)	Suckle → Feed 57 (44.31)

Figure 2. Chi-square analysis of relation between termination of a suckling bout and onset of an independent feeding bout ($df = 1$ for all values). Chi-square values appear in parentheses; N s appear above them for overall analysis, $\chi^2(1, N = 124) = 23.35, p < .0001$.

Experiment 2A: Behavioral Arousal Following Suckling: Postsuckling Behavioral Arousal in a Standardized Setting

Experiment 2 consisted of a series of procedures designed to describe the phenomenon of postsuckling behavioral arousal and to identify the components of suckling bout termination that trigger the arousal. First, in Experiment 2A we established that the phenomena observed in the naturalistic setting are replicable under standardized, manipulable conditions. In Experiments 2B and 2C, specific components of the nursing stimulus were systematically deleted to determine the cues that are necessary and sufficient for postsuckling behavioral arousal.

Pups in Experiment 2A were presented with an anesthetized dam from which the pups suckled and received milk.

After 20–25 min, the anesthetized dam was withdrawn. We compared subsequent levels of behavioral activity in experimental pups versus littermate control pups that had not been provided with a dam. We expected more behavioral arousal in the experimental pups.

Method

Subjects. For all studies, subjects were bred and housed as described in Experiment 1. For Experiments 2A and 2B the original stock was obtained from Charles River Labs, Inc. (Wilmington, MA); these animals were maintained on a 16:8 hr light–dark cycle. For the remaining experiments (2C, 3–5) original stock was obtained from Harlan Laboratories (Indianapolis, IN) and were maintained on a 12-hr light–dark cycle.

Six dams and their 20–22-day-old litters were used in Experiment 2A. Focal pups consisted of 10 males and 14 females.

Apparatus. For Experiments 2A–2C testing was conducted in a square compartment constructed of sheet metal (32.5 × 32.5 × 12.0 cm). Wood chips blanketed the floor. A clear Plexiglas lid covered the test cage. The lid was marked with lines approximately 0.5 cm wide that divided the cage into four equal quadrants.

Procedure. Twelve to 16 hr prior to testing, a litter was separated from its mother, divided equally between the two test cages, and deprived of food and water. Two pups in each cage were randomly selected as subjects and marked with an identifying pattern on their fur. The within-litter design controlled for variation in circadian rhythm as well as possible between-litter differences in baseline levels of arousal. The 12–16 hr pretest deprivation provided an acclimation period to the test environment.

Pups in the experimental condition (nipple with milk) experienced the composite of thermotactile, conspecific odor, nipple-associated oral cues, plus milk transfer involved in suckling. The test session was initiated with introduction of an anesthetized dam that provided milk to one of the 4-pup test litters (see below). The dam was placed supine with one side of the body touching the cage wall. The remaining half of the litter served as controls. For these subjects the cage lid was lifted and the bedding was stirred to control for the disturbance associated with introducing a stimulus rat. The start of the test session was synchronized with the start of the nursing bout for the littermates. Experimental pups were given 5–10 min to attach to the dam's nipples followed by a 20–25-min simulated suckling bout. Pups in the control condition were left undisturbed for a comparable time. At the end of the simulated nursing bout, pups in the experimental condition were dislodged from the adult stimulus by pulling them away by their tail. Similarly, pups in the control condition received equivalent tail pulls.

Test sessions were recorded using time-lapse video recorder (Gyr Model TLC1400; 6:1 playback ratio) starting at the beginning of the test period and lasting until 50 min following termination of the nursing bout. Behavioral arousal was measured during playback as total frequency of line crosses for the 2 focal pups in each test cage during the 50-min time period following the nursing bout.

Adult stimulus: Nipple with milk condition. After removal of her litter, each dam was given a foster litter of 4 pups comparable in age to her own litter. Approximately 6 hr before the test, the foster pups were removed. The foster litter was used to maintain nipple distention and avoid excessive accumulations of milk during the 12–16 hr pretest separation.

Immediately prior to testing, the dam was anesthetized (ketamine 90%, xylazine 10%, 0.14 ml/100 g, IM). A 3-cm longitudinal incision was made along the base of the tail to expose the

ventral vein. After puncturing the vein, a PE-10 cannula (Adams Clay) was inserted about 1 cm into the vein and secured with sutures 0.5 cm rostral and caudal to the opening. The incision was then sutured closed. The cannula was attached to a 1-ml syringe that contained oxytocin (Sigma Chemical) in 0.9% saline (1.5 IU/100 ml). Following surgery, the dam was placed supine in the test cage with one side of the body touching one of the walls. The dam's tail exited out of a small triangular opening at the bottom of one wall of the cage, thus isolating the cannula from the pups.

After all pups had attached (within 5–10 min), a series of seven injections of oxytocin, 3 min apart, was administered. The occurrence of pups' stretch responses and nipple shifting were recorded. Dosage was varied from .05 ml to .08 ml depending on previous response, that is, if the majority of the litter showed neither nipple shifting nor stretch responses to an injection, the next dosage was increased by .01 cc. Only pups that showed a minimum of four nipple shifts and stretch responses were included as subjects. This criterion resulted in the exclusion of 2 litters. To verify that this method resulted in milk transfer, 4 litters tested in groups of four were voided and then weighed before and after the simulated nursing bout. Mean weight gain was $1.25 \text{ g} \pm 0.10 \text{ g}$ per pup.

Following the seventh infusion, pups were dislodged from the dam's nipple by tail pull as described earlier and the dam was removed from the test cage. The cannula was clipped close to the incision and the dam was returned to her cage.

Reliability and data analysis. For Experiments 2A–2C interrater reliability scores for line crosses were within 94.1% of each other for repeated viewing of eight test sessions. For all experiments (2A–2C), mean frequency of line crosses per cage was calculated from the total frequency of line crosses for each pair of focal pups. Data for these experiments were analyzed using two-tailed matched-sample *t* tests.

Results and Discussion

Following removal of the dam, pups in the nipple with milk condition displayed more line crosses relative to control pups; their activity included play-fighting, self-grooming, as well as running about the test cage. In marked contrast, control pups remained relatively inactive following the stimulation of a tail pull, but without withdrawal of a nipple. Panel A of Figure 3 shows that mean frequency of line crosses for pups in the nipple with milk condition was 35.7 ± 4.4 , and mean frequency of line crosses for pups in the control condition was 10.9 ± 2.0 . This difference between the groups was significant, $t(5) = 4.20$ $p = .0088$. Thus, behavioral arousal followed termination of the suckling bout. The procedures used here provide a standardized, manipulable setting for further analysis of postsuckling behavioral arousal.

Experiment 2B: Behavioral Arousal Following Suckling: Is Milk Transfer Necessary for Postsuckling Behavioral Arousal?

In Experiment 2B, we examined suckling bouts without milk transfer to determine whether milk transfer is necessary to induce behavioral arousal. In this experiment, we compare the mean frequency of line crosses by pups that had suckled on an anesthetized dam but received no milk,

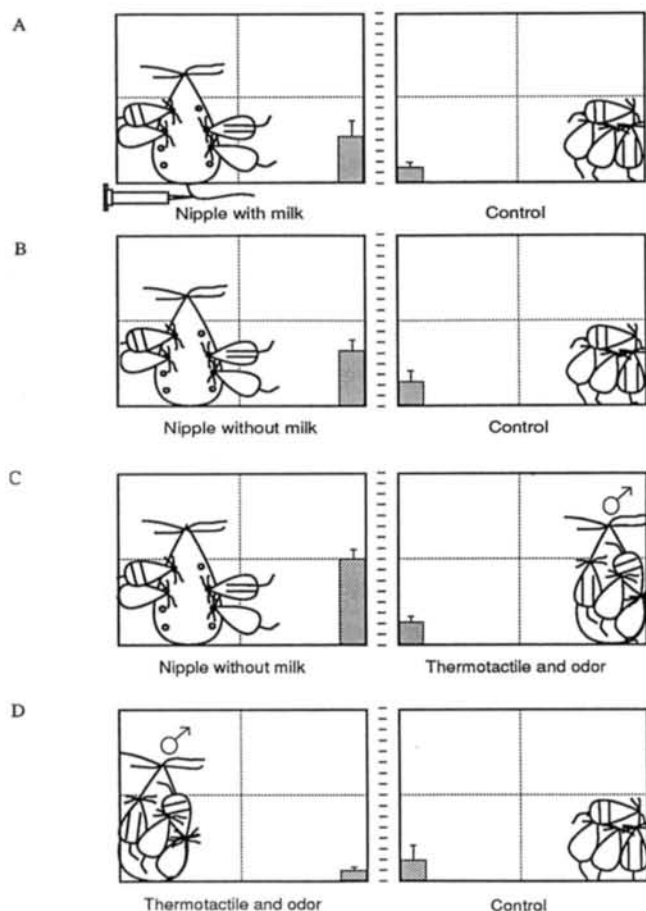


Figure 3. Summary of experimental manipulations and results for Experiments 2A–2C (refer to text for description of each manipulation). The vertical axis on each figure represents mean frequency of line crosses. The horizontal axis indicates each condition. Lines drawn on pups identify focal pups. Each dash is equivalent to an increment of 8.

with locomotion levels of controls that did not experience the dam at all.

Method

Subjects. Eight dams and their 20–22-day-old litters were used in this experiment. Focal pups consisted of 17 males and 15 females.

Procedure. Twelve to 16 hr prior to testing, litters were removed from the home cage, divided into an experimental (nipple without milk) and control group, and placed in identical test cages, as in Experiment 2A. Pups in the experimental condition were given a dam (see next section) that provided thermotactile, conspecific odor, and nipple-associated oral cues but did not provide milk. Milk transfer was blocked by anesthesia. The procedure was otherwise the same as described in Experiment 2A.

Adult stimulus: Nipple without milk condition. As in the previous experimental condition, dams were provided with a litter of 4 pups for the time that their own pups were absent until 6 hr before the test. This small litter suckled and received milk from the

dam. The dams were anesthetized as described in Experiment 2A, but did not receive surgical treatment.

Results and Discussion

Pups in the nipple without milk condition were significantly more active during the 50 min postsuckling test than were controls, $t(7) = 3.30$ $p = .013$. Figure 3B shows that pups in the experimental condition made a mean frequency of 42.9 ± 7.3 line crosses, whereas pups in the control condition made an average of 18.8 ± 7.7 line crosses. Pup activities included the same range of solitary and social behavior observed in Experiment 2A. Milk transfer is not necessary to induce behavioral arousal.

Experiment 2C: Behavioral Arousal Following Suckling: Is Withdrawal of Thermotactile and Olfactory Cues Sufficient to Potentiate Behavioral Activity?

When a dam leaves the litter after nursing, cues other than those directly associated with the teat are also withdrawn. The dam's thermotactile cues are reduced. Similarly, pups likely experience reduction of some of the dam's odor cues when she separates from the group.

There were two parts in Experiment 2C. We first compared pups' responses to withdrawal of an adult that provided suckling stimulation (but no milk) to withdrawal of an adult male rat that did not provide suckling stimulation. In the second part, we assessed behavioral arousal following removal of a nonsuckled stimulus, comparing it to the null condition.

Method

Subjects. Eight dams and their 20–22-day-old litters were used in the first part of this experiment. Focal pups consisted of 15 males and 17 females. An additional 8 males served as experimental stimuli. In the second part, testing involved 8 litters 20–22 days old; 20 females and 12 males served as focal pups. An additional 8 males were used as experimental stimuli.

Procedure. The basic procedures were identical to those used in the earlier experiments, except that the pretest deprivation phase was shortened. Six hours prior to the test, litters were divided in half, placed in identical test cages, and deprived of food and water. During the test in the first part of Experiment 2C, half of the litter had access to an anesthetized dam (see Experiment 2B, nipple without milk condition) to suckle; there was no milk transfer. The remaining half of the litter was presented with an anesthetized male (see next section) that provided the thermotactile and conspecific odors but obviated suckling (thermotactile and odor cues condition).

In the second part of this experiment, half of the litter was presented with an anesthetized male, as in the first part, and the remaining half of the litter spent the pretest phase as an aggregate and were stimulated by the tail pull procedure prior to the 50-min video test.

Adult stimulus: Thermotactile and odor cues condition. The adult males were anesthetized as described in earlier experiments. The pups huddled reliably with the anesthetized male. Neverthe-

less, pilot observations indicated that pups required more time to huddle with the anesthetized male than they did to attach to an anesthetized female. Therefore, pups in this group were allowed 20 min to establish contact with the male before the 20–25 min contact bout was terminated with tail pull stimulation and removal of the male. One litter that did not huddle with the male within the 20-min period following introduction of the adult male was excluded.

Results and Discussion

Withdrawal of an anesthetized dam that provided suckling stimulation but no milk induced more behavioral arousal in pups than did withdrawal of an anesthetized male, $t(7) = 6.42$, $p = .0004$. Figure 3C shows that nipple withdrawal resulted in a mean frequency of 65.4 ± 7.0 line crosses, whereas withdrawal of thermotactile and olfactory cues resulted in a mean frequency of 17.1 ± 3.8 line crosses.

Pup activity following withdrawal of the anesthetized male that provided conspecific olfactory and thermotactile cues was equivalent to that seen during the control condition. Specifically, removal of the anesthetized male led to a mean frequency of 7.6 ± 1.9 line crosses, and activity in the control pups averaged 15.4 ± 9.9 line crosses in the same period, $t(7) = -0.80$, $p = .45$ (see Figure 3D). Thus, both conditions produced equivalently low activity scores. Although it might be tempting to conclude that the withdrawal of peri-oral stimulation instigates behavioral arousal, it is possible that the reduction of some specifically maternal olfactory cues may be necessary to arouse pups. The use of the anesthetized male in the present experiment does not eliminate this possibility.

We therefore ran an additional experiment involving lactating females that did not provide suckling stimulation as the withdrawn stimulus. It is not trivial to prohibit deprived weanling-age pups from attaching to nipples of an anesthetized dam (hence, the use of males). To prevent pups from suckling but allowing contact, as well as access to maternal odors, these dams were fitted with a screen-mesh vest that slipped over the head and covered the nipples. The midline of the jacket was open, allowing direct thermotactile contact. Ventral edges of the jacket were secured to the dam's fur with collodion to prevent subjects from nosing under the opening and subsequently attaching. We included a control for effects of the presence of a mesh jacket. Control dams wore mesh vests modified to expose four nipples to which the pups attached.

Following removal of dams with accessible nipples there was greater activity by pups than after withdrawal of a dam with inaccessible nipples, $t(7) = 4.02$, $p = .005$. Following contact with nipples there was a mean frequency of 57.4 ± 7.6 line crosses, whereas after contact without nipples there were 27.8 ± 9.3 line crosses.

Frequencies of line crossing were equivalent in pups following removal of dams with inaccessible nipples and in control pups that simply received a tail pull, $t(7) = -0.43$, $p = .68$. Specifically, there was a mean of 17.3 ± 5.3 line crosses in pups previously housed with a dam with inacces-

sible nipples; pups in the control condition displayed a mean frequency of 20.6 ± 6.5 line crosses.

Together, the results of the various portions of the present experiment indicate to us that removal of peri-oral stimulation at the end of a nursing bout is a robust instigator of behavioral arousal in these weanling-age pups. Milk transfer is not necessary to stimulate behavioral arousal; nipple withdrawal alone is sufficient. Furthermore, there was no evidence indicating that a maternal odor or thermotactile cues were specifically involved. The present results support the idea that behavioral activation following a nursing bout is related to the withdrawal of peri-oral stimulation associated with having suckled on a nipple.

Experiment 3: Potentiation of Specific Behavioral Categories During Postsuckling Arousal

In the present experiment we again examined behavioral arousal in weanling rat pups after termination of contact with an anesthetized adult. Experiment 3 was designed to provide a more comprehensive and detailed picture of the postsuckling behavioral activity defined in Experiments 1 and 2. Our previous measure of locomotor behavior (line-crosses) was augmented in the present study by quantification of play behavior, self-grooming, social grooming, and sleep.

The adult stimulus animal in the present experiment provided either suckling without milk or suckling with milk transfer, thus allowing us to investigate behavioral arousal following milk transfer. Previously, Gerrish (1994) examined whether the addition of milk transfer to nipple withdrawal might facilitate behavioral arousal. Gerrish (1994) directly compared mean frequency of line crosses in pups either presented with an anesthetized dam that provided suckling with milk transfer or presented with an anesthetized dam that provided suckling but did not yield milk transfer. Results indicated no significant differences in line crosses between the two groups (pups were tested with both 12–16-hr and 6-hr food deprivation).

We retained the milk and no-milk conditions for the present experiment, despite the lack of evidence that milk transfer alters the pups' locomotor activity. Ingestion of fats and lactose in mother's milk might alter the quality of the aroused behavior, and this possibility was worth special attention. In addition, it seemed possible that we may have previously encountered a ceiling effect on linear movements and thus failed to see a contribution of the nutritive component of suckling.

Method

Subjects. Twelve dams and their 20–22-day-old litters were used. Focal pups consisted of 27 females and 21 males. Twelve additional dams served as stimuli.

Procedure. Six hours prior to the test litters were divided in half, placed in identical test cages, and deprived of food and water. During the test, half of the litter was presented with an anesthetized dam that provided the stimulus of nipple with milk transfer followed by its withdrawal (milk condition), and the remaining half

of the litter was presented with an anesthetized dam that provided the stimulus of nipple withdrawal and no milk transfer (nipple without milk, or no milk condition) and its subsequent withdrawal. Pups were given 5–10 min to attach to the dam's nipples followed by a 20–25 min nursing bout. Following termination of the nursing bout (tail pull and removal of the dam) a postsuckling observation period began. Videotaping began at the start of the test and lasted until 50 min after the dam was removed. For a detailed description of the procedure, see the *Method* section of Experiment 2A. For a description of preparation of the adult stimuli, see the *Adult stimulus* sections, nipple with milk condition in Experiment 2A and nipple without milk condition in Experiment 2B.

We examined a composite of pup behaviors in two focal pups during the 50 min following termination of a suckling bout. To facilitate these observations, the video record:playback ratio was 1:1. During playback, we measured the duration, or frequency and duration of the following behaviors: (a) sleep was pups resting with eyes closed (duration); (b) self-groom was licking and grooming any part of the body with the forepaws (duration); (c) social-groom was licking and grooming any part of a littermate's body (duration); and (d) play-fighting was rough-and-tumble play (this was the predominant pattern), chasing, and biting (frequency and duration).

Data analysis. Data were analyzed using two-tailed matched-sample *t* tests. Contrary to our expectations three pups in the milk condition slept for a relatively large portion of the test session. Specifically, these pups spent an average of 75.2% of the test period asleep or resting. Of all remaining 21 half-litters in both conditions, none slept for more than 44.0% of the test period, and the average proportion of time spent sleeping was considerably less, only 22.6%. Therefore the combined data from these 3 litters was removed from statistical analysis (i.e., both the milk and no milk condition, to include the matched controls) because their overall activity was limited.

Reliability. A single observer twice scored three videotaped sessions. Reliability was then calculated two ways. First we calculated an intrarater correlation (Pearson's) value between repeated scoring of the three tapes. The correlation incorporating all duration measures (includes sleep, self-groom, social-groom, and play fighting) was .999. The correlation for play duration alone was .997, and play frequency was .958. Next, we used a more conservative assessment. For each measure we calculated a level of congruence, using percentage agreements (agree/agree + disagree \times 100) for duration and frequency measures. On average, the duration measures were within 95% of each other. Play durations agreed within 94% of each other and play frequencies agreed within 85.1%.

Results and Discussion

Pups tested in the milk condition played more than those in the no milk group (Figure 4). For example, pups in the milk condition showed a mean duration of 102.6 ± 15.6 s of play, whereas pups in the no milk condition showed a mean duration of 46.7 ± 10.2 s of play during the 50-min observation period. These differences were statistically significant, $t(8) = 3.48$, $p = .008$. Mean frequency of play bouts was 28.7 ± 2.8 for pups in the milk condition and 14.7 ± 2.1 for pups in the no milk condition, $t(8) = 4.94$, $p = .001$. Thus, play was more robust in pups that received milk compared with those that did not. Comparison of self-groom durations indicated that pups in the milk condition also showed more self-grooming than did pups in the no milk condition, 298.6 ± 30.3 and 203.8 ± 19.7 , respectively, $t(8) = 2.67$, $p = .028$. Mean duration of social groom

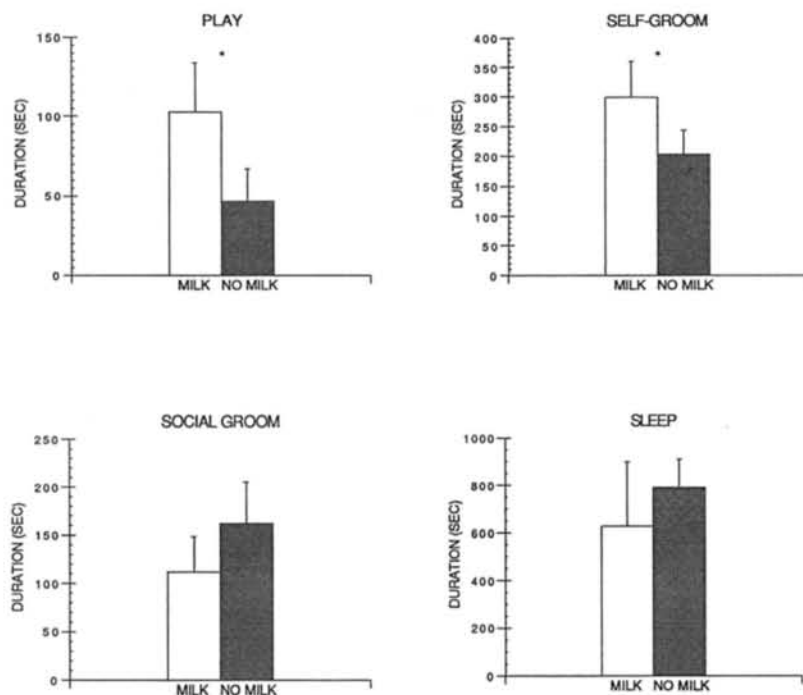


Figure 4. Duration of play-fighting (play), self-grooming (self-groom), social grooming (social-groom), and sleep for pups in the milk and no-milk conditions. Sec = seconds.

was 111.8 ± 18.5 for pups in the milk condition and 162.2 ± 21.6 for pups in the no milk condition, $t(8) = -1.70$, $p = .128$. Finally, there were no significant differences in duration of time spent sleeping. Mean sleep duration was 627.4 ± 135.6 for pups in the milk condition and 790.5 ± 59.4 for pups in the no milk condition, $t(8) = -.901$, $p = .40$.

Although we eliminated those pups and their matched controls that slept during the majority of the test period, we were interested in examining the resulting behavioral patterns and including these outliers in the data set. Results for play approached significance, indicating a trend consistent with data reported above. Pups in the milk condition showed a mean duration of 80.5 ± 16.4 s of play, and pups in the no milk condition showed a mean duration of 47.1 ± 9.6 s of play during the 50-min observation period, $t(11) = 1.87$, $p = .088$. Mean frequency of play bouts was 22.8 ± 3.7 for pups in the milk condition and 15.1 ± 2.6 for pups in the no milk condition, $t(11) = 1.76$, $p = .106$. However, there were no differences between the groups in durations of self-grooming or sleep. Pups in the milk condition spent a mean duration of 246.3 ± 35.6 s self-grooming and pups in the no milk condition spent a mean duration of 217.3 ± 18.9 s self-grooming, $t(11) = .641$, $p = .54$. Mean duration for sleep was 1034.2 ± 238.1 s for pups in the milk condition and 715.8 ± 67.6 s for pups in the no milk condition, $t(11) = -1.1$, $p = 1.1$, $p = .30$. Interestingly, duration spent social grooming was greater for pups in the no milk condition, 165.1 ± 19.0 s, compared with those in the milk condition, 95.0 ± 16.8 s, $t(11) = -2.50$, $p = .03$. Recall that without these three pairs of litters, there was a trend toward greater social grooming in the no milk condition. Frequently, when play occurs it is preceded by social grooming. Thus, one manifestation of this behavioral arousal appears to be that pups that have recently received milk are more apt to express greater arousal by extending social grooming into play-fighting.

These data suggest a bifurcation of responses for pups in the milk condition. The computer-tabulated durations of the behaviors as well as notes taken during observations of the milk group indicated that these pups were either very active or they appeared to be asleep for virtually the entire duration of the test period. Examination of the standard errors of the mean when all pups are included in the data analysis (see values in preceding paragraph) indicates that pups in the milk condition compared to the no milk condition generally showed greater variability in all behaviors measured except for social grooming. When the three litters that slept were removed from the dataset, the variability in the milk group was reduced (as discussed earlier in associated text; see also Figure 4), supporting the notion that recent milk consumption may stimulate two different behavioral outcomes—behavioral arousal or sleep. However, behavioral arousal, particularly expressed as play, is more likely. In contrast, pups in the no milk condition were more homogeneous in their responses. It is possible that the two different behavioral responses to milk consumption might reflect amount of milk consumed. To prevent arousal due to handling, we did not measure weight gain following milk transfer.

Thiels et al. (1990) reported that around the time of weaning, self-grooming, play-fighting, and feeding occurred independently from the dam. Thus, in the context of the present study, the functional significance of increased play from milk transfer is that it represents greater independence from the dam, greater overall arousal, and therefore an enhanced likelihood of nest egression, exploration, and ingestion of solid food.

Together, these analyses indicate significantly greater behavioral arousal following the stimulation of both nipple and milk, compared to nipple stimulation alone. This difference was not revealed by simple measure of line crosses. A finer analysis, including the composite of behavioral responses following nursing, yielded a more complete and revealing depiction of behavioral events following the various treatments. These results suggest that the enhanced behavioral arousal displayed by the majority of pups in the milk condition may be related to the additional caloric energy available after milk transfer.

Experiment 4: A Preliminary Consideration of Postsuckling Thermogenesis

We have described a general response in rat pups to the mother's departure after a nursing bout: Pups display surges of behavioral arousal, manifested as increased locomotion (Experiment 2) and potentiated play behavior (Experiment 3). Such postsuckling arousal appears to function as an antecedent to early feeding bouts (Experiment 1).

The weaning process in rats seems to be propelled by certain predictable, but rather nonspecific events, such as those that support the egression from the nest by weanlings and that bring them into the vicinity of foods. A crucial variable in this general formula for weaning is temperature. We have demonstrated that ambient temperature affects how long pups stay outside the insulative confines of the maternal nest and, consequently, how much they explore and initiate activities from which feeding derives (Gerrish & Alberts, 1996). Experiment 4 was designed as a preliminary inquiry into a distinctly different aspect of thermal influences on weaning. Pups almost always lose heat to the environment, especially when they are moving independently outside the nest. The effect of temperature on exploration and weaning is likely related to some differential between ambient temperature and the pup's temperature, which, in turn, is related to the pup's rate of heat production (as well as its rate of heat loss).

Postsuckling surges in activity and exploration, particularly as they are affected by milk intake (Experiment 3), suggest the possibility that there is a physiological correlate of behavioral arousal following milk transfer. For example, there might be an increase in metabolic heat production, or a suckling-induced thermogenesis, due to energy obtained from milk. Indeed, even the furred, weanling-aged pups are vulnerable to cool temperatures. During early nest egressions they might benefit from increased heat production. Hence, we measured oxygen consumption (heat production) in suckled versus nonsuckled, weanling-aged rat pups. Ox-

xygen consumption was measured both at a warm temperature known to produce minimal metabolic activity (30 °C; Conklin & Heggeness, 1971) and then at a temperature (21 °C) that presented a mild cold challenge. We predicted that suckled pups would show a higher resting metabolic rate than nonsuckled pups at either or both of these temperatures.

Method

Subjects. Subjects were 16 pups, 6 males and 10 females, each from a different litter, 20–22 days old. Mean body weight was 51.14 ± 1.11 g for the 14 subjects actually used in the data analysis (see *Data analysis* section). Refer to the *Method* section in Experiment 1 for breeding and housing information.

Apparatus. Individual rat pups were tested in a metabolic chamber that consisted of a double-walled glass cylinder (17.5 cm high \times 9.0 cm wide). The top end was fitted with a rubber stopper that could be removed to insert an animal holding cage (described later). The sides of the chamber were equipped with air inlet and outlet ports. A temperature probe was fed through the inlet port. Air temperature inside the chamber was regulated by a water bath (Lauda RM6, Germany) that circulated heated or cooled water between the walls of the chamber. An advantage of the glass-walled cylinder was that pup behavior was readily visible.

Oxygen consumption was measured with a dual channel oxygen analyzer (Ametek S-3A, Pittsburgh, PA). A tank of certified compressed dry air (20.94–21.00% oxygen) directed two airstreams to the respiratory apparatus. One stream passed through a digital flowmeter (Digi Flow 200) to deliver 400 ml/min of dry air into the animal chamber (described in the *Procedure* section). The line exited the animal chamber through a tube of desiccant and was drawn (95–120 ml/min) through one cell of the oxygen sensor. There was a 2-cm diameter opening in the effluent line prior to the sensor that permitted excess air to escape. Because the sensor drew only a fraction of the air passing through the metabolic chamber, the opening served pressure-relief, compensating for fluctuations in effluent flow, such as those caused by animal movements. The second airstream from the tank was drawn at the same rate (95–120 ml/min) directly to the second cell of the oxygen sensor. Oxygen inside the oxygen sensor was heated to ionization temperature in independent electrochemical cells. The system thus provided a continuous digital display of the difference in oxygen between cells one and two of the sensor. The output of the oxygen analyzer and a thermocouple probe placed inside the chamber was read by a Macintosh SE computer programmed (Omegalog, Stamford, CT) to provide at 1-min intervals a record of oxygen consumed and temperature inside the chamber.

Procedure. Subjects were placed in a maternity cage with their littermates and deprived of the dam, food, and water for approximately 6 hr before testing. Pups were randomly assigned to a suckled or nonsuckled condition. Pups in the suckled condition were voided, weighed, and then returned to the dam along with their littermates about 50 min prior to testing, giving them ample time to settle and attach to their mother's nipples and receive milk. Pups in the nonsuckled condition were voided and weighed, but then returned to the deprivation cage with their littermates. Following this 50-min period, pups in both conditions were weighed a second time.

Following the suckling or nonsuckling interval, an individual subject was placed in a small compartment (7.0 \times 5.5 \times 3.5 cm) constructed from a polyethylene mesh and then put into the cylinder (described earlier), which served as the test chamber. The

polyethylene cage was raised approximately 2.4 cm from the bottom of the chamber. The size and design of the polyethylene chamber minimized movement and prevented direct contact between the animal and the inside surface (floor and sides) of the chamber. Once the animal chamber was introduced into the metabolic chamber, the stopper was replaced and the system ran for 25 min to stabilize pressure, flow, and temperature.

Starting temperature was approximately 30 °C (within the thermoneutral range of 21-day-old pups; Conklin & Heggeness, 1971). After the 25-min stabilization period, baseline resting metabolic rate was collected for 10 min. Ambient temperature was then decreased to approximately 21 °C to provide a mild cold challenge (it took approximately 10 min for the temperature to drop from 30 °C to 21 °C). Pups remained in the test chamber for 1 hr from the time of the cooling period.

Data analysis. For each subject we calculated mean rates of oxygen consumption (ml O₂/kg/min) during the 10 min prior to start of the temperature drop and from the time that the chamber reached 21 °C until the rat was removed (typically 50 min). Body weight used in the calculations was the initial weight measurement, that is before pups suckled or were further deprived. Data were analyzed separately for the 30 °C and 21 °C temperatures, using two-tailed, two-sample *t* tests.

Two subjects were deleted from the subject pool, one in each condition, because of unstable baseline rates of oxygen consumption. Stability was defined as a range less than or equal to 10 ml/kg/min for 10 consecutive min during the baseline recording at 30 °C.

Results and Discussion

Suckled pups' oxygen consumption in the warm (30 °C) condition was greater than that of the nonsuckled pups. At a thermoneutral temperature (30 °C), suckled pups consumed $38.79 \pm .83$ ml O₂/kg/min, whereas the nonsuckled pups used $36.27 \pm .73$ ml O₂/kg/min. This difference was statistically significant, $t(12) = 2.27$, $p = .04$. Oxygen consumption rates at 21 °C indicated a trend toward greater heat production by suckled pups than by nonsuckled pups, but the difference was not statistically significant, $t(12) = 1.73$, $p = .11$. During the cold challenge, rate of oxygen consumption was 67.75 ± 1.28 ml/min/kg for suckled pups and 64.63 ± 1.27 ml/min/kg for nonsuckled pups.

We confirmed milk transfer by examining body weights: Mean weight gain for pups in the suckled condition was $1.09 \text{ g} \pm 0.27 \text{ g}$, and mean weight loss for pups in the nonsuckled condition was $0.19 \text{ g} - 0.18 \text{ g}$. Correlations between weight gain and oxygen consumption rate at both temperatures for pups in the suckled group were not significant: 30 °C, $r(7) = -.011$, $t(5) = 0.024$, $p > .25$; 21 °C, $r(7) = .247$, $t(5) = 0.568$, $p > .25$. This indicates that the pre-suckling body weights were valid to use because amount of milk consumed was not directly related to greater heat production.

Thus, we found that the suckled pups' rate of metabolic heat production was 6.9% greater than that of the nonsuckled pups at a nonchallenging temperature. Though the difference was small, it was statistically significant. The suckled pups' 4.8% increment over nonsuckled pups in the cool temperature approached, but did not achieve, statistical significance.

The present results indicate that the transfer of mother's milk can augment the weanling's metabolic heat production within minutes of ingestion. Because the effects measured in this experiment were modest, we were not able to gauge the overall behavioral, physiological, or developmental significance of the phenomenon. There are several reasons to suspect, however, that the observed phenomenon might reflect a much larger thermogenic effect of milk ingestion. First, we tested pups individually and the absence of social stimuli might have attenuated some of their physiological and behavioral responses. It is possible, for example, that there may be a kind of feed-forward mechanism whereby milk enhances physiological heat production as well as behavioral activity. The muscular component of behavioral arousal, which was essentially eliminated in the present study, can also be a source of physiological heat. Limitations on the pups' activity may well have diminished their total thermogenic effort normally triggered by suckling with milk transfer. Finally, the particular temperature regime used in testing for the present experiment may have interacted with the pups' overall response. The initial warm, thermoneutral temperature may have partially sedated them or modified their responses to the cooling phase. It is likely that both extent and duration of temperature exposures can interact with behavioral and physiological responses.

In light of such complexities, we view the present results as a useful indication that milk transfer may have a behaviorally meaningful, physiological effect on heat production. One possible form of such an effect is that pups become warmer, and that this warmth provides a form of emancipation from the nest, enabling them to venture into the outside world further and for longer, thus exposing them to new stimuli that augment the weaning process.

Experiment 5: Postsuckling Behavioral Activation in Preweanling Pups

The purpose of Experiment 5 was to determine whether the behavioral arousal following a nursing bout termination is displayed by pups younger than the weanlings used in the previous experiments. If postsuckling arousal is characteristic of pups only at weaning onset, then the behavior might be considered a mechanism specifically related to the onset of independent feeding. In the present study we compared behavioral arousal in 10- to 12-day-old pups following nipple withdrawal and no milk with activity by nonexposed littermate controls.

Method

Subjects. Eight dams and their 10- to 12-day-old litters were used in this study. There were 15 male and 17 female focal pups.

Procedure. The procedure was generally the same as described for older subjects in the *Method* section of Experiment 2A, with modifications intended to accommodate for the smaller size, greater nursing requirements, and limited motor capabilities of the subjects.

Younger pups suckle more frequently than weanling-aged pups. Therefore, test litters were separated from the dam, divided in half,

and placed in identical test cages for only 2.5 hr prior to the test. The test cage was a standard maternity cage, but a sheet metal divider placed in the center of the cage reduced the area to $9.5 \times 7.5 \times 6.5$ cm. This division allowed just enough space to place the dam in the cage during the nursing bout. During the test, half of the litter was presented with an anesthetized dam that provided the stimulus of nipple without milk (see the *Adult stimulus: Nipple without milk* section of Experiment 2B). The remaining half of the litter served as controls. Pups in the experimental condition were given 5–10 min to attach to the dam's nipples, and pups in the control condition remained undisturbed for an equivalent amount of time. Sometimes the young pups in the experimental condition appeared to be asleep when the dam was placed in the cage; in such an instance the pups were placed near the dam and actively guided toward a nipple. Pups in both conditions were given a 20–25 min nursing bout. At the end of the nursing bout pups in both conditions received a tail pull and, in addition, the dam was removed from litters in the experimental group. Then, a small metal corner piece was placed around the pups, and they were gently pushed until the metal corner piece reached the corner of the maternal cage, thus creating a small box around the pups ($11.5 \times 11.5 \times 10$ cm). This reduced the activity space of the pups so that we essentially observed movement within the huddle and thus accommodated for pups' smaller size and stage of motor development. A clear Plexiglas lid was placed on top of the box. Two lines approximately 0.5 cm wide divided the box into four equal quadrants. Following the tail pull, marking termination of a suckling bout, the postsuckling observation period was video recorded. Videotaping began at the start of the test. During playback we measured frequency of line crosses for 2 focal pups in each condition for 50 min after termination of the nursing bout.

Data analysis. Line crosses were calculated for each cage as the mean frequency of total line crosses per pair of focal pups. Data were analyzed using two-tailed matched-sample *t* tests.

Results and Discussion

In every litter tested, pups presented with a dam showed a considerably higher mean frequency of line crosses than did the controls. Pups in the nipple without milk condition had a mean frequency of 58.9 ± 5.2 line crosses, whereas pups in the control condition had a mean frequency of 15.5 ± 2.3 line crosses. Statistical analysis indicated that mean frequency of line crosses was significantly greater for pups in the experimental as compared to the control condition, $t(7) = 11.26$ $p = .0001$. These results indicate that arousal following nursing is not specific to weanling pups just beginning to ingest solid food. These results do not support the notion of the existence of a developmentally specific mechanism in the 20- to 22-day-old pup. Rather, 10- to 12-day-old pups' behavioral repertoire leads to a different expression of behavioral arousal. It is not until the pups develop the host of features associated with leaving the nest that suckling-induced behavioral arousal becomes functionally important for the weaning transition.

General Discussion

Diminishing sufficiency of mother's milk as a nutrient source does not explain weaning onset in rat pups (Thiels et al., 1988). The present series of experiments provide a

foundation for understanding how specific behavioral events and interactions contribute to weaning onset. In Experiment 1, we found a relation between suckling and feeding: There is a close correlation between termination of a suckling bout and the onset of independent feeding bouts. Furthermore, termination of suckling bouts is often followed by a dramatic flurry of pup activity. Withdrawal of the nipple and milk transfer stimulate behavioral arousal, whereas removal of thermotactile cues, and conspecific and lactating dam olfactory cues do not (Experiments 2 & 3). Thus, at weaning onset, nipple withdrawal and milk transfer lead to a burst of activity resulting in egression from the nest and ingestion of solid food.

The present results illuminate a somewhat surprising characteristic of the weaning process. It appears that many of the key events that propel weaning are typically not recognized as components of ingestive behavior, yet they function in the transition from ingesting milk to ingesting food. We found that generalized behavioral arousal, play behavior, exploration, and some nonnutritive (albeit oral) stimuli are functional antecedents of early feeding in rat pups.

Pup behavior is activated or increased by events in the nest associated with the termination of nursing bouts. Postsuckling activation, expressed by the weanling, creates a syndrome of intense activity with intensified social interactions, including play. We also observed postsuckling arousal in 10- to 12-day-old preweaning pups (Experiment 5). After suckling, they move more and more rapidly around the nest, and huddle vigorously within the litter. Behavior beyond the huddle and nest is not part of their typical repertoire. Thus, postsuckling activation does not appear to be developmentally specific to the weanling, but the kinds of behavior that are activated in the weanling-age rat make a specific contribution to weaning.

We note a convergence of observations regarding associations between play and weaning. Bateson and his associates, using analytic approaches different than ours, have described conditions that both increase play and accelerate weaning in kittens (*Felis catus*; Bateson, Martin, & Young, 1981; Bateson, Mendl, & Feaver, 1990; Bateson & Young, 1981, Martin & Bateson, 1985). Smith (1991) reported findings in Norway rats resembling those of Bateson and colleagues.

In the present series of experiments, we have identified developmental processes that contribute to weaning by sending pups from the nest and into the vicinity of solid food. For example, play behavior literally moves the pup around its environment, and undoubtedly increases events considered to be egression from the nest. Play and generalized behavioral arousal enhance the likelihood that pups will leave the nest and spend more time out in the field and encounter solid food. Environmental temperature is also an important influence on nest egression. Food is typically located outside the warmth of the nest. Initial nest egressions and first feedings on solid food are delayed if the temperature outside the nest is cold (Gerrish & Alberts, 1996). Thus, the pups' ability to maintain thermal homeostasis is a necessary condition for initial nest egressions

and for maintaining their presence around food. It is possible that postsuckling thermogenesis may increase the duration of nest egression and postsuckling exploration (Experiment 4).

Once outside the nest, there are factors that guide the pups' activities in the direction of solid food, a source of sustenance that will soon be vital to their existence. Weanlings are known to be attracted to adults that are in the vicinity of solid food (Galef & Clark, 1971). In the absence of adult conspecifics weaning is delayed (Galef, 1971). Residual odors from conspecifics are also known to attract pups to a food site (Galef & Heiber, 1976). Once in the vicinity of food, the pup is more liable to examine and to sample substances that bear chemosensory resemblance to familiar, mother-associated cues (Galef & Henderson, 1972; Galef & Sherry, 1973). At that point, powerful mechanisms of learning support the rapid acquisition of associations between food cues and postingestive events related to the presence of nutrients in the sample (e.g., Melcer & Alberts, 1989).

Thus, given the appropriate thermal milieu, an integral part of the actual transition to feeding appears to derive in a rather general way from postsuckling activation. This arousal directs the young away from the nest and brings them into to contact with various social and chemical cues that guide them toward food and promote sampling. It is not the case that the pups feed to allay an energy deficit due to insufficient milk (cf. Thiels & Alberts, 1985). Behavioral factors seem to be vital to instigating feeding (Thiels et al., 1988). Indeed results from the present study indicate that pups feed independently after they have just received milk, suggesting that they are not ingesting solid food because of hunger. They are, instead, aroused and active as a consequence of a predictable and regular aspect of mother-litter interactions—termination of a suckling bout.

Perhaps it should not be considered highly surprising that these general mechanisms shape a specific event such as weaning to solid food. Hall and Williams's (1983) compelling argument that suckling and independent feeding are two separate systems allows us to consider that the processes described in the present report serve to move the weanling from one system to the other, rather than transform one specific form of ingestion into another form.

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Implementing the Guidelines for the Ethical Treatment of Animals

The Committee on Animal Research and Ethics (CARE) is a standing committee of the American Psychological Association, charged with reviewing and recommending guidelines for the ethical treatment of animals in research and teaching, disseminating these guidelines, and consulting in their implementation. Requests for help in interpreting and implementing the guidelines should be addressed to the Research Ethics Officer, Science Directorate, American Psychological Association, 750 First Street, NE, Washington, DC 20002-4242; phone: (202) 336-6000; fax: (202) 336-5953; e-mail: science@apa.org