

Regina A. Abel

April E. Ronca

Jeffrey R. Alberts

Department of Psychology
Indiana University
Bloomington, IN 47405

Perinatal Stimulation Facilitates Suckling Onset in Newborn Rats

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ABSTRACT: *The fetus' experience of birth derives from a sequence of stimulation provided by the mother's labor contractions, her licking and handling, and the contrasting environmental conditions of the uterus and outside world. In the present investigation, Day 21 fetal rats were externalized from the dam's body; subjects in one uterine horn were compressed by simulated uterine contractions while control subjects in the opposite horn were not compressed. All pups were Cesarean-delivered, stroked, and exposed to a thermal environment simulating either room (21°C), nest (33°C), or intrauterine (36°C) temperature. After 1-hr exposure to the experimental temperature, all pups were maintained at 33°C and tested for their suckling response to an anesthetized dam. When newborns were tested at 120 min postpartum, simulated contractions increased the probability of nipple attachment in pups exposed to 21°C relative to noncompressed littermates maintained at the same temperature. Atypically warm postpartum conditions (nestlike or intrauterine) obviated the effects of compression by increasing suckling above the levels seen in noncompressed newborns exposed to the cool condition. Thus, compressions facilitate the achievement of suckling under thermal conditions resembling those typically encountered by the newborn rat.* © 1998 John Wiley & Sons, Inc. *Dev Psychobiol* **32**: 91–99, 1998

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To become a newborn, a mammalian fetus must make numerous and varied transformations. The present research expands and elaborates the perspective that the birth process itself, beginning with the mother's labor contractions, provides organizational factors for the fetus-to-newborn transition. Breathing and suckling are two vital behavioral adaptations of the newborn. Sensory stimulation associated with birth facilitates respiratory behavior (e.g., Condorelli & Scarpelli, 1975; Gluckman, Gunn, & Johnston, 1983; Ronca &

Alberts, 1995a, 1995b; Scarpelli, Condorelli, & Cosmi, 1977). In the present investigation, we examined the roles of mechanical stimuli during labor contractions and thermal stimuli after birth in the initiation of suckling by the newborn rat pup.

Labor contractions provide mechanical forces that help push fetal mammals from the warm fluid world of the maternal uterus through the birth canal and into the cool, gaseous external environment. The contractions of a mother's abdominal and uterine muscles are impressive in number and in strength. Norway rats' labor contractions appear as three distinct types of movements and postures: lordosis contractions, vertical contractions, and peristaltic waves (Dollinger, Hol-loway, & Dennenberg, 1980; Rosenblatt & Lehrman,

Correspondence to: R. A. Abel
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1963). Of the three types, lordosis contractions occur most frequently, and fetuses receive an average of 84 such contractions in the 6 hr before birth (Ronca, Lamkin, & Alberts, 1993). An intrauterine balloon, inflated to the size of a fetus and attached to a pressure transducer remote from a free-moving dam, indicates that these contractions exert forces on the fetuses averaging about 10–15 mm Hg (Fuchs, 1969; Higuchi, Uchida, Honda, & Negro, 1987).

The dam assists each pup's exit from the birth canal, detaches the pup's placenta and umbilical cord, and licks the newborn, thus removing birth membranes and fluids. These activities are completed before the next pup emerges (Rosenblatt & Lehrman, 1963). The "interpup interval" during vaginal births is about 10 min, so delivery of an average litter spans about 90 min [range = 40–136 min (Ronca et al., 1993)]. During the parturition, newly born pups lie scattered in vicinity of the dam. Small, exposed, and still damp with maternal saliva and birth fluids, newborn pups cool rapidly. Infrared thermal imaging shows that in a 22°C environment, a newborn pup's body surface temperature decreases from 37.5°C to 25°C in about 15 min (Alberts, Ronca, & Blumberg, 1992). After the entire litter is delivered, the dam assembles the pups in the natal nest and positions herself over them, making her mammary region accessible for nursing (e.g., Ronca et al., 1993; Rosenblatt & Lehrman, 1963). The microenvironment of the nest, consisting of the mother's body, nesting materials, and littermates, provides a source of heat and insulation. Temperature within the nest is thought to be about 33°C (e.g., Leon, Croskerry, & Smith, 1978). Suckling begins after the pups' body surfaces have warmed to 31–33°C (unpublished observations).

Despite extreme immaturity of their sensory apparatus, fetal rats can detect mechanical stimuli of the type and magnitude present in utero, including that which occurs during labor and delivery (Ronca & Alberts, 1994). The *experience* of such stimulation appears to influence profoundly the fetus' transition to newborn (Ronca & Alberts, 1995a, 1995b). Without the experience of the mother's labor contractions and/or licking, fetuses failed to initiate continuous postpartum respiration even when challenged by hypoxia associated with umbilical cord occlusion. Respiratory movements in unstimulated pups virtually ceased within the 1st hr following delivery. Breathing was successfully established by pups that were stimulated prenatally with compressions simulating labor contractions and postnatally with strokes to the body surface that simulated maternal licking. These studies provide an empirically based explanation of Pedersen and Blass' (1982) observation that without postnatal

stroking, mortality rates among their Cesarean-delivered rats was 40%. Thus, the *experience of birth stimuli* appears essential to the transition from fetus to newborn. But this perspective has only been applied to studies of the onset of breathing.

The purpose of the present study was to examine the effects of prenatal compressions and ambient temperature on the establishment of suckling by the newborn rat. Briefly, fetuses were externalized from the mother's body and exposed to a sequence of compressions delivered with a device designed and calibrated to mimic labor contractions. Pups were then Cesarean-delivered and administered tactile stimulation resembling maternal licking. We manipulated postpartum temperature by using one of three biologically relevant temperatures. Newborns were exposed to a cool room-temperature environment (21°C), or to a warmer surround maintained at nest (33°C) or intrauterine (36°C) temperature. After 1 hr postpartum exposure to one of the three experimental temperature manipulations, pups from all groups were placed at nest temperature, and then tested for nipple attachment 90 min and again at 120 min postpartum. The 21°C condition contained the sequence of thermal exposures experienced by a vaginally born rat pup under typical conditions. This treatment regime, then, was designed to represent the sequence and duration of stimulation that normally occurs prior to and immediately after vaginal birth, leading to the onset of suckling.

Behavioral tests of newborns appeared feasible: In preliminary observations we separated pups from the mother immediately after completion of a normal, uninterrupted vaginal birth. Pups were moved into a 21°C environment for 1 hr and then, at 90 and 120 min after the beginning of the temperature exposure, they were given a nipple attachment test on an anesthetized dam. In the initial test performed at 90-min postpartum, 50% of the 10 pups attached to a nipple. After 2 hr, 90% attached (Abel, Ronca, & Alberts, 1996). It appeared that we could use such a test to detect alterations in the onset of suckling under well-controlled conditions that match the basic parameters of the normal, vaginal birth.

METHODS

Subjects

Sixty perinates from 10 rat (*Rattus norvegicus*) dams were subjects. Female Sprague-Dawley rats (80–100 days), bred in the Indiana University colony, were time-mated to Sprague-Dawley males. Pregnant dams

were housed in standard polyurethane maternity tubs in a colony room maintained at 22°C with 12 : 12 light: dark cycle (lights on 0800 hr). Purina Rat Chow and water were available ad lib.

Procedure

Treatment of Pregnant Rat Dams. On gestational Day 21 (G 21; day of conception = gestational Day 0, day of birth = gestation Day 22) pregnant dams were anesthetized by placing them individually into a bell jar containing 2 cc isoflurane (Aerrane, Ohmeda PPD Inc., Liberty Corner, NJ). After the rats were appropriately anesthetized (approximately 1–2 min), a small (3 cm) incision was made over the lumbar region. Chemomyelotomy was performed by inserting a needle (30 ga \times $\frac{1}{2}$) between L1 and L2 and injecting 0.10 ml ethanol (100%). The dam was then placed in a Plexiglas holding device, and her lower body immersed into a warm (37.5°C \pm .5°C) buffered saline bath (Narayanan, Fox, & Hamburger, 1971; Smotherman, Richards, & Robinson, 1984). A midline laparotomy was performed and the uterine horns were externalized into the bath.

Treatment of Fetuses. Three contiguous fetuses (beginning with the fetus located second from the ovary) from each uterine horn were used as subjects. One uterine horn was gently lifted and each subject fetus, still encased within the horn, rested against the fingertips of the researcher. A small, inflated latex balloon attached to a gram scale (Health o meter No. 1031533) was pressed against each of the 3 fetuses in succession. Compressions of 150- to 170-g pressure on the hand-held scale [approximating pressure measured during actual labor contractions (Ronca & Alberts, 1994)] were administered at the rate of one 15-s compression per min for 10 min for each of the 3 subjects. Fetuses in the opposite uterine horn were noncompressed controls. Immediately following the 10 compressions, subject fetuses from both horns were individually delivered onto moist gauze pads (1-ml isotonic saline/pad). Birth membranes were removed using two cotton-tipped swabs to tear open the amniotic sacs and to clear membranes away from neonates. Umbilical cords were tied with surgical silk and removed. Neonates were then placed onto moist gauze pads in individual 235-ml plastic cups (Rubbermaid Model 0018) where they remained throughout the experiment. Compressed and noncompressed horns were counterbalanced across dams.

Treatment of Newborns. One compressed subject and a noncompressed littermate were assigned to each of

three postnatal temperature conditions: intrauterine (36°C), nest (33°C), or ambient room temperature (21°C) ($n = 10$ subjects/group). Intrauterine- and nest-temperature neonates were placed in incubators heated to the appropriate temperature. To insure that independent respiration was successfully established, both compressed and noncompressed pups were vigorously stroked with a soft-bristled No. 3 artist's brush (approximately 2–3 min/litter). Strokes were applied with gentle pressure across the full length of the body; pups were turned with the brush to permit stroking on both sides of the body. All subjects were maintained at the experimental temperature until 60-min postpartum. Neonates in the intrauterine- and ambient-room groups were then moved to the nest-temperature incubator for an additional 30 min; subjects in the nest-temperature group were handled in the same manner but were not exposed to a change in temperature. All subjects remained in individual containers at 33°C until 90-min postpartum.

Nipple-Attachment Test Procedure. A recently parturient (1–2 days postpartum) dam was anesthetized with ketamine (ip; 100 mg/ml, 0.9 ml/kg) and xylazine (ip; 20 mg/ml, 0.5 ml/kg) and placed in a supine position in a 33°C test incubator approximately 20 min prior to test. Beginning at 90 min postdelivery, each pup was placed with its snout in contact with a nipple of the test dam and gently held in contact with the dam's ventrum for a 2-min nipple attachment trial (Pedersen & Blass, 1982). Successful attachment was verified when the pup maintained grasp of the nipple when gently pulled away from dam, and time was recorded for the determination of attachment latency. A score of 120 s was assigned to all pups that failed to attach.

Following the first nipple-attachment trial, neonates remained in the 33°C incubator until 120-min postpartum, and the same nipple-attachment procedure was followed for the second trial for pups in 9 of the 10 litters.

Data Analyses

The test for significance of difference between two proportions (Bruning & Kintz, 1968) was used to analyze difference in the proportion of compressed and noncompressed newborns that successfully attached to nipples. To analyze the temperature effect, attachment frequencies for compressed and noncompressed subjects in each temperature condition were combined, and an overall chi-square analysis was performed for both the 90- and 120-min tests.

The distribution of latency scores was truncated by

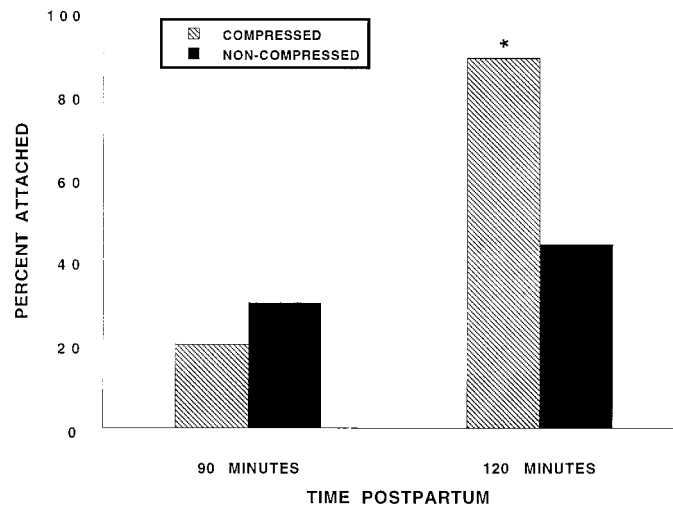


FIGURE 1 Percent of pups in the room-temperature condition that attached to the nipples of anesthetized dam at 90-min (left) and 120-min (right) postpartum. *indicates $p < .01$ (test for significance of difference between two proportions).

the 2-min limit used for the attachment test. Therefore, a log transformation was performed on latency scores to stabilize the variances (Winer, 1971). Log scores were then analyzed using a repeated measures analysis of variance (ANOVA). Appropriate individual comparisons were made on latency scores using Scheffe’s post-hoc test (Howell, 1992).

RESULTS

Both prenatal compression and postpartum temperature affected nipple attachment by the newborn pups. The most dramatic effects of prenatal compression were seen between pups that experienced thermal conditions similar to those of normal, vaginally delivered pups (i.e., the room-temperature condition), whereas thermal effects were most evident in pups exposed to atypically warm temperatures (i.e., the intrauterine-temperature condition).

A significant effect of prenatal compression was observed during the test at 120-min postpartum, in which 89% of pups that had received prenatal compression attached to nipples, whereas only 44% of the noncompressed littermates attached and suckled, $z = 3.26, p < .01$. Figure 1 shows the percent of both compressed and noncompressed pups in the room-temperature conditions that successfully attached in tests performed at both 90- and 120-min postpartum. At 120 min there was an apparent enhancement of suckling by compressed pups that had been exposed to a 21°C environment; it was here that the deficit of the noncompressed pups was revealed (see Figure 1).

Prior to the 120-min test, relatively few pups in the room-temperature condition attached to nipples during the initial test conducted after 30-min exposure to nest temperature. Specifically, 20% of the prenatally compressed pups and 30% of noncompressed pups attached when tested at 90-min postpartum, $z = .74, p > .10$.

Table 1 enumerates the performance of all the groups in nipple attachment tests performed at both 90 and 120 min. At 90 min, 70% of pups that were maintained at intrauterine temperature (36°C) attached to the nipples of the test dam, regardless of their experience of compression. Within the nest-temperature condition, frequency of nipple attachment was relatively low, and there was no discernible effect of prenatal compression at 90 min. Compressed pups and

Table 1. Percent of Pups Attaching to Nipples of Anesthetized Dams

Group	90 Min		120 Min	
	% Attached	N	% Attached	N
Intrauterine (36°C)				
Compressed	70	10	89	9
Noncompressed	70	10	78	9
Nest (33°C)				
Compressed	40	10	56	9
Noncompressed	20	10	67	9
Room (21°C)				
Compressed	20	10	89*	9
Noncompressed	30	10	44	9

*indicates significance $p < .01$. (Test for significance of difference between two proportions).

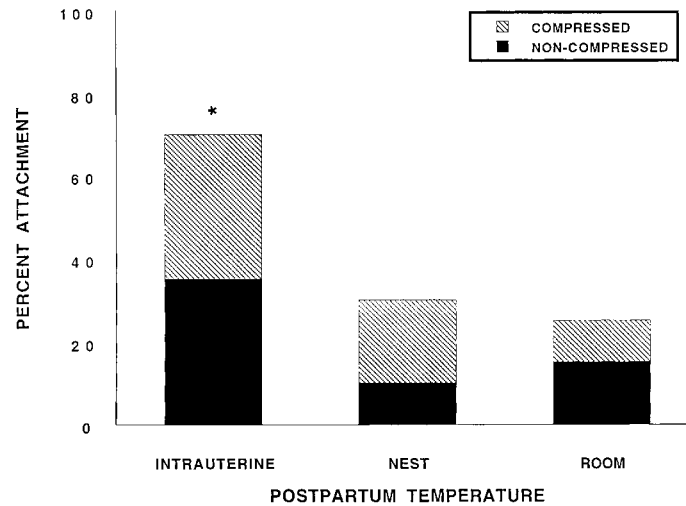


FIGURE 2 Total percent of pups that attached to the nipples of an anesthetized dam at 90-min postpartum. *indicates $p < .02$ (chi-square test).

noncompressed pups exposed to nest temperature (33°C) attached to nipples in similar proportions, 20% and 40%, respectively, $z = 1.43$, $p > .10$. When tested at 120-min postpartum, nipple-attachment percentages for warm-compressed and warm-noncompressed pups were 89% and 78%, respectively, $z = .90$, $p > .10$; attachment percentages for pups in the nest-compressed and nest-noncompressed conditions were 56% and 67%, respectively, $z = .68$, $p > .10$.

There was an overall effect of temperature on the pups' behavior in tests performed at 90 min. As shown in Figure 2, when data for compressed and noncompressed pups were combined within each temperature condition, we found that frequency of nipple attachment was lower at the lower temperatures, $\chi^2(1) = 6.40$, $p = .012$. At 120 min, however, attachment frequencies were equivalent in the intrauterine- and nest-temperature groups, respectively, $\chi^2(1) = .40$, $p > .50$, n.s.; $\chi^2(1) = .234$, $p > .60$, n.s.

We also performed an additional set of evaluations using latencies to attach to nipples. An overall analysis of variance on log transformations of attachment latency scores indicates a significant effect of compression, $F(1) = 6.43$, $p < .02$, and of postpartum time, 90 and 120 min, $F(1) = 13.15$, $p < .01$, as well as a significant Compression \times Postpartum Time interaction, $F(1) = 4.81$, $p < .04$. Table 2 contains mean attachment latencies for all groups. Post-hoc analysis indicated there was no difference in attachment latency between compressed and noncompressed subjects at 90-min postpartum. At 120 min, depicted in Figure 3, attachment latency for compressed subjects in the room-temperature condition was significantly less than for noncompressed subjects in the same temperature condition, Scheffe $F = 6.72$, $p < .05$. There were no significant differences in attachment latency between compressed and noncompressed pups in either the intrauterine- or nest-temperature conditions.

Table 2. Mean Latency to Nipple Attachment (Seconds)

Group	Latency at 90 Min	<i>N</i>	Latency at 120 Min	<i>N</i>
Intrauterine (36°C)	Mean (<i>SEM</i>)		Mean (<i>SEM</i>)	
Compressed	77.4 (12.66)	10	48.49 (10.63)	9
Noncompressed	73.6 (11.73)	10	72.78 (11.78)	9
Nest (33°C)				
Compressed	93.4 (11.19)	10	69.56 (16.16)	9
Noncompressed	111.5 (7.69)	10	79.78 (12.28)	9
Room (21°C)				
Compressed	90.27 (7.16)	10	45.33 (11.41)*	9
Noncompressed	93.7 (6.71)	10	91.56 (12.32)	9

*indicates significance $p < .05$ (Scheffe test).

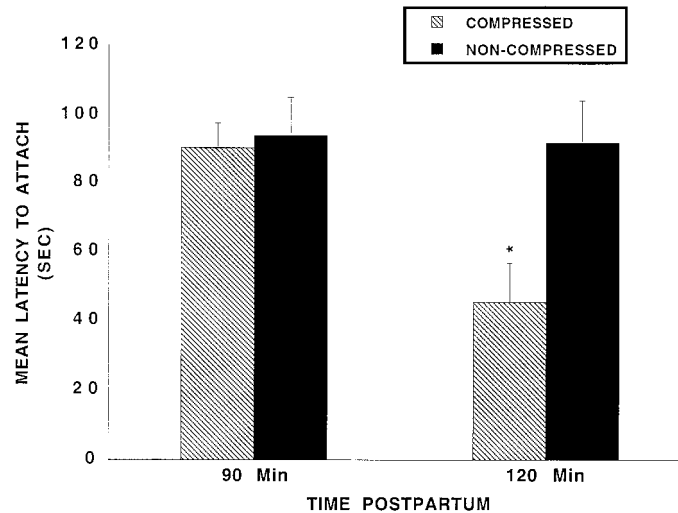


FIGURE 3 Latency (seconds) to nipple attachment for pups in the room-temperature condition at 90-min (left) and 120-min (right) postpartum. *indicates $p < .05$ (Scheffe post-hoc analysis).

The robust, statistically significant effect of compression on frequency of nipple attachment (Figure 1) combined with the effect on attachment latencies (Figure 3) reinforce our view that pups do indeed attach to nipples more readily if they have experienced compressions simulating labor contractions.

DISCUSSION

The main, novel behavioral finding of the present investigation is that prenatal compression facilitates onset of suckling for pups exposed to a postnatal temperature regime similar to that experienced following normal, vaginal delivery. Cesarean-delivered fetuses that experienced body compressions that mimic the dam's labor contractions, followed by a period of stroking that mimics maternal licking, attached with greater frequency to the nipples of an anesthetized dam than did noncompressed (but "licked") littermates.

Labor contractions have been linked to onset of breathing in newborn rats (Ronca & Alberts, 1995a, 1995b), but other, more-complex behavioral patterns have not been examined this way. Vaginally delivered infants exhibit both enhanced respiratory performance and increased alertness compared to Cesarean-delivered infants whose mothers did not undergo full labor (Hagnevik, Lagercrantz, & Sjoqvist, 1991; Hjalmarsson, Krantz, Jacobsson, & Sorensen, 1982; Otamiri, Berg, Ledin, Leijon, & Lagercrantz, 1991; Ushers, Allen, & Maclean, 1971). Catecholamine concentration, particularly that of norepinephrine, is higher in vaginally delivered human infants than in Cesarean-delivered infants (Jones & Greiss, 1982), and there is a

relationship between plasma catecholamine levels present at birth and both respiration and alertness (Faxelius, Hagnevik, Lagercrantz, Lundell, & Irestedt, 1983; Lagercrantz & Bistoletti, 1977; Lagercrantz & Slotkin, 1986; Otamiri et al., 1991). Together, these observations suggest that "birth stimuli," i.e., the range, levels, and patterns of stimulation that comprise the birth process, might have multiple roles in the successful transition from fetal to postnatal life (Ronca, Abel, & Alberts, 1996).

We have shown that labor contractions do more than move a fetus through the birth canal. Whether by design (selection) or by incidental effect, contractions provide a form of stimulation which serves to facilitate at least two neonatal achievements: pulmonary respiration and suckling. There is growing recognition that the catecholamine surge that accompanies labor is integral to successful newborn status.

We think it is prudent to use the term *facilitation* to denote that labor contractions affect already-ongoing processes, namely the development of respiratory behavior and of suckling in the neonate. It seems erroneous to suggest that compressions initiate or "trigger" either behavior. First, mammalian fetuses produce breathing movements throughout the latter half of gestation (Liggins, 1982). Fetuses also show coordinated mouth and tongue movements (Narayanan et al., 1971) as well as nipple capture (Robinson, Hoeltzel, Cooke, Umphress, & Smotherman, 1993). Thus, suckling behavior does not appear to arise de novo in the newborn. Finally, some pups in the noncompressed group attached to nipples, which seems to indicate that compressions are not necessary for nipple attachment. (The reader can consult Gottlieb, 1976, for a discus-

sion that was seminal to our perspective on experiential mechanisms.)

The role of ambient temperature in first nipple attachment appears significant but difficult to define precisely at this point. Without prenatal compressions, 2 hr of postnatal exposure to a warm ambiance (33–36°C) facilitated nipple attachment (see Figure 2 and Table 1). Does warmth accelerate the same processes that compression facilitates? Does prenatal compression work on the perinate by augmenting heat production so that the newborn can better warm itself and commence suckling? The mechanism(s) by which thermal variables participate in organismic function require further analysis.

There were no obvious gross behavioral differences between groups at the time of testing. Newborns in all groups were initially inactive during the immediate postdelivery period, lying on one side with body relaxed and limbs loosely extended. Within a few minutes following introduction to the warmer temperatures, pups often displayed cyclic episodes of body and limb movements and stereotypic treading, followed by a curled posture and then righting to a prone position. Mouthing frequently accompanied locomotor activity as pups began moving about their individual containers. Pups in the room-temperature condition typically remained on their sides throughout the hour of cool exposure and were, for the most part, immobile. However, as subjects that had been exposed to room temperature began to warm following introduction to nest temperature, they appeared to produce the same types of motor behavior exhibited by subjects in the warmer conditions.

Immediately preceding nipple-attachment tests, any differences in motor abilities were not readily discernible. Pups were active and, upon introduction to the anesthetized mother, characteristically scanned the dam's ventrum. Pups in the noncompressed, room-temperature group, however, appeared more likely to rest their snout in contact with the nipple or to scan over the nipple without orienting to it. Specificity of compression effects on perinatal motor behavior is yet to be determined.

It is noteworthy that the temperature conditions that make prenatal compressions functionally significant for suckling are the temperature conditions normally experienced by vaginally delivered newborns. Perinates in the room-temperature groups were removed from the warm amniotic surround, delivered into a cool outer environment, and were further stimulated while birth membranes were removed. After an hour of exposure to 21°C, a vigorous challenge to the small neonate, pups were rewarmed as they would be following normal delivery. Previous observations using

thermal imaging techniques indicated that newborns required 45 min or more to rewarm to nest temperature following exposure to the cool, extrauterine environment (unpublished observations). Compressions may not be necessary for the early expression of suckling under atypically warm conditions, but contractions do augment suckling in pups that have been cooled and rewarmed.

Mechanical stimuli, the sort derived from compressing and stroking the perinate's body, also facilitate breathing in newborn rats (Ronca & Alberts, 1995a, 1995b). We therefore considered whether nipple attachment by pups in the room-temperature group may have been compromised by inferior respiratory status during the tests. In a separate study (unpublished) Cesarean-delivered pups, both compressed and noncompressed, were exposed to the same postnatal stroking and temperature regime used in the present study. (For purposes of that study, 50 μ l saline was injected into the amniotic fluid of each subject.) One-min-long samples of respiration frequency were taken at the time of nipple-attachment tests. Average respiratory frequencies (cycles per min = cpm) of pups in the two conditions were not significantly different: 77 cpm ($SE = 4.9$) in compressed pups and 82.3 cpm ($SE = 3.6$) in noncompressed pups. These values compare favorably with measures of the respiration frequency of 68 cpm ($SE = 6.2$) measured in vaginally delivered pups. There was no evidence of delayed or compromised respiration contributing to differences in nipple-attachment frequencies. We were further encouraged to conclude that the present methods supported good respiratory onset when we noted excellent agreement between the present results and those of our previous studies of respiratory development following stroking or compression (Ronca & Alberts, 1995a, 1995b).

Stimulus specificity in this developmental setting is unresolved. Though we make a procedural distinction between compression and stroking, it has not been shown that the perinate responds differentially to such stimulation. These forms of stimulation may be interchangeable in the developmental processes we have examined. Compression and stroking are equivalent in promotion of respiration (Ronca & Alberts, 1995a, 1995b); therefore, additional stroking may substitute for compressions, and more or stronger compressions might reduce the amount of stroking stimuli needed for attaining independent respiration and nipple attachment. These possibilities seem consistent with the variability seen in natural birth sequences. Birth order within a litter of 10–12 pups, for example, will expose the early emergers to fewer contractions but to more licking and greater temperature flux than that imping-

ing on later pups that are probably compressed more, but exposed to fewer early postnatal stimuli (see also Ronca et al., 1993).

The establishment of pulmonary ventilation and the onset of suckling are behavioral milestones of the mammalian fetus' transition to newborn. In addition to identifying a previously unrecognized regulation in the fetus-to-newborn transition, the present study provides a preparation by which perinatal stimulation can be manipulated with relatively well-defined, controlled, and validated methods. These methods can be applied to more systematic analyses of the perinate's remarkable transformations.

NOTES

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