Human Newborns Prefer Human Milk: Conspecific Milk Odor Is Attractive Without Postnatal Exposure

Luc Marlier and Benoist Schaal
Centre National de la Recherche Scientifique, France

Behavioral responses of 3- to 4-day-old newborns to the odors of various human milk (HM) and formula milk (FM) were examined in paired-choice tests. When both stimuli were nonfamiliar, breast-fed, as well as bottle-fed, infants oriented their head and mouthed more vigorously to HM than to FM. When breast-fed infants were exposed to nonfamiliar HM along with the familiar FM, their head-turning responses were undifferentiated although they mouthed more frequently to the human stimulus. When nonfamiliar HM and familiar FM were equalized in intensity, nonfamiliar HM again elicited more head orientation and mouthing responses. These results demonstrate that the odor of HM is more attractive to human newborns than FM and that this preference is independent of postnatal feeding experience.

Human newborns react to the odor emitted by the breasts of lactating conspecifics. Early support for this claim was raised by newborn infants’ refusal to grasp orally a nipple previously anointed with a repulsive odorant whereas the other olfactorily intact nipple was accepted without delay (Preyer, 1881). Experiments conducted from 1975 to 1980 led to more decisive results demonstrating that human newborns detect and recognize the odor of their mother’s breast within the first days of life (Macfarlane, 1975; Russell, 1976; Schaal et al., 1980). These initial data were later enriched by studies that investigated the conditions that led to the establishment of breast odor preferences in newborns. Specifically, any artificial odorant associated with breast feeding rapidly gained reinforcing power (Cernoch & Porter, 1985; Schleidt & Genzel, 1990). The general supposition was made that these same associative processes could explain the spontaneous development of preferences for natural odors from the maternal breast.

However, differential responsive behavior to odor stimuli has been noted right after birth, before infants have had extensive postnatal exposure to them. For example, Steiner (1979) noted that from less than 12 hr of age, before any ingestion, newborns displayed distinctive facial expressions that evidenced emotional responses to hedonically extreme (to adults) odorants. Another approach confirmed that bottle-fed infants showed preferential head orientation to the breast odor of nonfamiliar lactating women (Makin & Porter, 1989). As these infants had in principle never been in direct contact with lactating breasts, it may be suggested that their attraction was not based on postnatal experience.

Several factors may operate in the early development of such a selective odor repertoire and of its cognitive incorporation shortly after birth. First, neonates may rely on previous odor acquisitions, as the fetal brain can already store odor information and use it in directing postnatal actions (Hepper, 1995; Marlier, Schaal, & Soussignan, 1998a; Mennella, Jagnow, & Beauchamp, 2001; Schaal, Marlier, & Soussignan, 1995, 1998, 2000). Second, newborns may be attracted to given odor cues independently of any previous direct exposure to them. Such unconditional odorants have been recently documented in several mammals (Blass, 1990; Schaal et al., 2003; Smotherman & Robinson, 1992).

As the preceding categories of acquisition mechanisms operate simultaneously during normal development, their respective contributions to the initial expression of preferences is not easy to isolate. One way to reduce the complexity of causal

Participating mothers and infants are warmly acknowledged for their interest and patience. We are also grateful to the director, midwives, nurses, and technicians of the Clinique Sainte-Anne (Strasbourg, Alsace, France) for their constant support. We acknowledge valuable discussions with A. Holley, R. Soussignan, F. Kopp, E. Lehr, C. Mathis, A. Ofinowski, and F. Valence. Finally, we thank S. Guimier for her help in videotape coding, and C. Mori and A. Lester for their comments and suggestions on an earlier draft of the manuscript.

Affiliation of the authors: Centre des Sciences du Goût, UMR 5170, CNRS, UB, INRA, Dijon, France.

Correspondence concerning this article should be addressed to Luc Marlier, Clinique Sainte-Anne, Maternité, 182 Route de la Wanzenau, 67000 Strasbourg, France. Electronic mail may be sent to marlier@cesg.cnrs.fr.

© 2005 by the Society for Research in Child Development, Inc.
All rights reserved. 0009-3920/2005/7601-0011
analyses is to assess neonatal responses to stimuli to which perception was denied during one stage of development. To meet such an aim, we examined the responsiveness of infants who did not have direct exposure to a lactating mother’s breast or milk (i.e., they were exclusively bottle fed since birth) when presented with the odor of human milk (HM).

In the present experiments we pursued four goals. First, we assessed the stimulating effect of HM odor on neonatal behavior. Second, we compared the behavioral activity of HM odor in infants who were normally exposed to the breast during feeding with that in infants who have never been exposed to a lactating breast because they were exclusively bottle fed. Third, we weighed the reinforcing power of the odor of conspecific (human) milk against that of an artificial formula based on bovine milk both to assess whether every mammalian milk odor is attractive to infants and to measure the relative attraction strength of different types of milks as a function of direct experience with them. Finally, we examined the preceding three goals using multiple variables that encompass several dimensions of an infant’s behavior, such as (a) general orientation indicative of stimulus attention and seeking, and (b) oral actions as markers of appetite and ingestion readiness.

<table>
<thead>
<tr>
<th>Main experiments</th>
<th>Control experiments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Experiment n</td>
<td>1 2 3 4</td>
</tr>
<tr>
<td>Infants (n)</td>
<td>12 12 12 10</td>
</tr>
<tr>
<td>Females (n)</td>
<td>6 6 7 4</td>
</tr>
<tr>
<td>Males (n)</td>
<td>6 6 5 6</td>
</tr>
<tr>
<td>Gestational age at birth (weeks)</td>
<td></td>
</tr>
<tr>
<td>M</td>
<td>38.9 39.8 39.5 39.5</td>
</tr>
<tr>
<td>SD</td>
<td>1.1 0.8 1.2 1.2</td>
</tr>
<tr>
<td>Range</td>
<td>37.5–41 38.5–41 37.5–41 38–41</td>
</tr>
<tr>
<td>Mode of delivery (n) vaginal/caesarean</td>
<td></td>
</tr>
<tr>
<td>10/2</td>
<td>11/1 10/2 8/2</td>
</tr>
<tr>
<td>Birthweight (g)</td>
<td></td>
</tr>
<tr>
<td>M</td>
<td>3,342 3,358 3,263 3,367</td>
</tr>
<tr>
<td>SD</td>
<td>3.46 371 301 539</td>
</tr>
<tr>
<td>Range</td>
<td>2,950–4,100 2,850–4,000 2,860–3,760 2,880–4,230</td>
</tr>
<tr>
<td>Firstborn (n)</td>
<td>6/12 4/12 4/12 6/10</td>
</tr>
<tr>
<td>Age at testing (h)</td>
<td></td>
</tr>
<tr>
<td>M</td>
<td>93.8 83.3 92.5 86.4</td>
</tr>
<tr>
<td>SD</td>
<td>21.2 12.3 15.8 15.2</td>
</tr>
<tr>
<td>Range</td>
<td>72–119 66–101 78–121 72–117</td>
</tr>
<tr>
<td>Feeding method (n) breast/bottle</td>
<td></td>
</tr>
<tr>
<td>12/0</td>
<td>0/12 0/12 0/10 8/0</td>
</tr>
</tbody>
</table>

General Method

Participants

The newborns were recruited in Clinique Sainte Anne (Strasbourg, Alsace, France) on Postnatal Day 4 (M age at testing = 88.4 h, SD = 16.9 h). The selection criteria included term delivery (i.e., gestational age: 38–41 weeks), absence of gestational or perinatal complications (Apgar score >8 at 1 min, and Apgar score = 10 at 5 and 10 min after expulsion), absence of neonatal pathologies or defects, and an exclusive feeding method (breast or bottle) since birth. The sample consisted of 67 White infants (34 boys, 33 girls) whose general characteristics as well as those of their mothers are given in Table 1. Mean maternal age at delivery was 29.8 (SD = 4.9) years.

Six independent groups of infants were drawn, which were homogeneous for gender, $\chi^2 = .015$, $p > .90$; gestational age, $F(5, 61) = 1.14$, $p > .30$; birth weight, $F(5, 61) = .29$, $p > .30$; and age at testing, $F(5, 61) = .65$, $p > .30$.

General Conditions

After obtaining written parental consent, the experimental phase took place in a quiet room of the maternity hospital. The temperature and light conditions were held constant (22–24°C). The tests were
performed in the morning (8:00 a.m. to 12:00 p.m.) on the spontaneous waking of the newborns (in states three or four of Prechtl’s, 1974, classification) and before a feed (M delay between last feed and test = 3.4, SD = 1.2 h). When stabilized in the required state, each infant was installed in a semireclining position in a seat designed for newborns (back set at a 25° angle with the vertical) and swaddled up to the axillae. In situations where the infants fell asleep, they were aroused by vocal stimulations and stroking of the palms. In contrast, when an infant became too aroused, the test was delayed until the next feed (this was the case for 18 of 67 infants). Each infant was tested once.

**Stimuli**

Two milk stimuli were used: HM and formula milk (FM). HM was sampled by manual extraction into plastic flasks (70 ml; sample volume range = 10–40 ml). Milk collection was done (under supervision by the experimenter) by the mother or the nurse on Day 4 postpartum. As breakfast remains homogeneous in composition within and between mothers, the milk samples were collected in the morning to minimize the influence of the mother’s diet on the variability of the milk aroma. Furthermore, as the dietary aromas pass into milk within 1 to 3 hr after intake (Mennella & Beauchamp, 1991), milk collection was performed within this period (M = 2.6, SD = 1.3 h after the end of the meal). To minimize potential alterations to the odor properties of the milk, the samples were either: (a) directly used in a test (n = 22) if the infant was in the right state; (b) immediately chilled (4°C; n = 15) if testing was scheduled for the next 2 hr; or (c) immediately frozen (−20°C) and then quickly thawed under lukewarm water in the minute preceding the test; frozen samples (n = 9) were used within 24 hr and were discarded after that time. Each sample was used in only one test.

FMs were ready-to-use formulas based on bovine milk given to bottle-fed newborns in the local hospital (brands: Alma, Gallia, Guigoz, Nidal, Nutricia).

The stimuli were presented on 100% cotton gauze pads (10 cm², Sterilux®, Harthmann, Châtenois, France). The pads were odorized by pipetting 20 drops of either milk in a standardized way. The control stimulus consisted of a gauze pad moistened with the same amount of distilled water using the same procedure. All stimuli were presented at ambient temperature.

**Odor-Presentation Device**

For the test, the infant was introduced into a test chamber (1 × 0.7 × 1 m) made of a light beige-colo-

![Figure 1. The device used for olfactory paired-choice tests. Two gauze pads (A and B) are fixed symmetrically on each side of the infant’s midline (eccentricity: 20-70 degrees; see text for more details).](image-url)
presentation. After the presentation of the second stimulus, the infant’s head was returned to the sagittal position and released after dissipation of the tonic neck asymmetry. The infant’s behavior was then video-recorded during a first 1-min trial. To control for a potential lateral bias of head turning, the side of the odor stimuli was reversed after the first trial so that the stimulus presented on the right was presented on the left, and vice versa. The infant’s head was then again oriented manually to each stimulus before being released in the midline and recorded for a second 1-min trial. A minimal interval period of 2 min was entered between both trials composing a test.

**Dependent Variables**

The video-recorded sequences were analyzed by a coder who was unaware of the nature and lateral position of the paired stimuli. The behavioral events were timed from a TV screen with the internal clock of the computer through Observer 2.0 software (accuracy: 0.1 s; Noldus, 1991). This software allows online analysis of predefined behavioral items. First, the duration of head turning to either stimulus was coded by tracking the tip of the infant’s nose over the 180° trajectory that it could scan. The orientation response to a stimulus was positive when the participant’s nose was positioned over the sector corresponding to that stimulus pad (i.e., when the nose deviated from the sagittal plane at an angle ranging from 20° to 70° to the right or left sides). The durations the infants spent oriented to a given stimulus zone were summed across the two consecutive trials composing a test. For each participant, the orientation duration to a given stimulus was reported as the proportion of the time spent turned toward that stimulus to the total time of observation (120 s). Eventual crying or yawning were excluded from the total duration of the test. This case occurred rarely, however (7 of 67 tests), and when it occurred the duration subtracted from the test never exceeded 6 s.

Second, several variables extracted from the head orientation analysis were used to characterize the general pattern of the infant’s cephalic activity in the paired-choice tests. The latency of first head turning was defined as the time between release of the head and the infant’s self-orientation to either stimulus. The amount of head activity over either stimulus was obtained by dividing the stimulus sector (20°–70°) in two equivalent sectors (represented by angles 20°–45° and 45°–70° from the midline) and by counting the occurrences of nose crossing of this criterion line (see dotted lines in Figure 1). This variable was used to evaluate the magnitude of search-like movements.

Finally, several movement patterns were subsumed under the terms oral activities or mouthing, including mouth opening with protruded lips, tongue protruding, licking, and sucking with or without contact with the hands. Oral motor activity linked with crying or yawning was not entered in the calculation. Newborns were considered to be expressing mouthing activities toward a given stimulus pad when their nose was simultaneously positioned over that stimulus. For each infant, the durations of oral activities were summed across the two consecutive trials composing the test. The level of oral activation induced by either stimulus was calculated by relating the total duration of oral activities to a given stimulus to the duration of nose orientation to that stimulus.

**Interobserver Reliability**

Reliability was assessed by comparing the data from two coders who independently timed 20 videotaped tests randomly selected among the different experiments. Both coders were blind to the nature of the stimuli presented, and one was unaware of the goals of the study. Spearman rank-order correlation coefficients were computed to assess their agreement regarding the head orientation and the mouthing variables. For the head orientation durations to either stimulus, the measures of both coders were highly correlated (Odor A, \( r = .95 \); Odor B, \( r = .97 \)). For oral activation measures, the correlations between both coders reached .88 and .93 for Odors A and B, respectively.

**Statistical Treatments**

To assess the differential effect of odor stimuli on the duration of head orientation in the experiments, two-way repeated measures analyses of variance (ANOVAs) were computed. The nature of the stimulus (Odor A vs. Odor B) and the side of presentation (right vs. left) were considered as potential determinants of the neonates’ head orientation responses. As previous work on neonatal responsiveness to odors using the present test situation did not reveal any effect of gender on head orientation (Marlier & Schaal, 1997; Marlier et al., 1998a, 1998b; Schaal, Marlier, et al., 1995, 1998), this factor was not considered here. To test the main and interaction effects revealed by the ANOVA, post hoc \( t \) tests for matched samples were conducted. The differential effect of either stimulus on oral activation was assessed using
tests for matched samples. To inspect the choice pattern expressed by individual neonates, the number of participants orienting longer, or expressing higher oral activation, to Stimulus A than to Stimulus B was compared with chance distribution (i.e., 50%) using the chi-square test. All statistical treatments were realized using Statistica 5.1 software (Statsoft, Tulsa, OK).

**General Results**

*Pattern of Head Turning in the Paired-Choice Test*

Different aspects of the infants’ responses were considered to assess whether the test situation was free of lateral biases and fatigue effects.

**Initial Lateral Bias**

The number of infants deviating first to the right side after the release of the head was compared with the number of infants deviating first to the left side, regardless of the nature of the stimuli presented. The data obtained in the four main experiments (Experiments 1–4) revealed neither strong nor consistent influence of the side of the stimulus on the infants’ initial head deviation (Table 2).

**Initial Directionality**

Are infants able to smell from the midline start position the difference between both stimuli and to orient to one stimulus at once? When the number of infants who oriented first to Stimulus A was compared with the number of infants who oriented first to Stimulus B, it appeared that the initial orientation was randomly distributed in all experiments (Table 2). This suggests that the newborns’ decision toward a given stimulus is established neither during the pretest exposure to both stimuli nor during the first positioning over a stimulus.

**Alternation of Head Movements**

We computed the number of alternations of the side of the nose across the midline of the test device and the number of entries of the nose over the stimulus sectors (Table 2). The mean number of bilateral head alternations ranged from 13 to 20 per test, and the infants positioned their nose an equal number of times over either stimulus (7–12 and 6–10 times for Stimuli A and B, respectively; cf. Table 2), attesting that either stimulus was reached and that the side alternation of both stimuli did not impede the participant’s olfactory choice.
Temporal Distribution of Head-Scanning Movements

The number of sectors covered by the infant’s nose was summated per periods of 15 s in Trials 1 and 2 that constituted a test (i.e., eight periods for a complete test). No significant difference was noted between the trials (Table 3), indicating that the infants displayed continuous head-scanning activity throughout the test.

In summary, in the present experimental conditions, the infants manifested an active exploration behavior throughout the test. Their movements revealed that they were trapped neither by a lateral bias of head movements nor by the initial stimulus encountered.

### Experiment 1

**Method**

Twelve breast-fed newborns (Table 1) were tested on Day 4 postpartum. They were simultaneously exposed to the odor of nonfamiliar HM (from a woman who was not the mother of the infant tested) and the odor of a nonfamiliar FM.

**Results and Discussion**

**Head Orientation**

*Relative duration of orientation.* A two-way (Odor × Side of Presentation) ANOVA revealed a main effect of the odor stimulus on the duration of head orientation, $F(1, 11) = 13.94, p < .01$. The newborns oriented longer to the odor of nonfamiliar HM ($M = 473, SD = .165$) than to the odor of a nonfamiliar FM ($M = .203, SD = .129$; Figure 2, column A). No additional main or interaction effects of the side of presentation reached significance. When individual differential responses were considered, 10 of the 12 neonates oriented longer to the odor of nonfamiliar HM than to the odor of nonfamiliar FM, $\chi^2(12) = 5.33, p < .05$.

*Latencies to reach either stimulus.* A two-way (Odor × Side of Presentation) ANOVA indicated a main odor effect on the latencies to reach either stimulus. When facing the testing device presenting both odor stimuli, breast-fed infants directed their head more quickly toward the nonfamiliar HM (right: 9.8 ± 18.4 s; left: 9 ± 17 s) than toward the nonfamiliar FM (right: 23.9 ± 23.7 s; left: 23 ± 23.6 s), $F(1, 11) = 10.49, p < .01$. No main effect of the side of presentation or interaction effect between this factor and the nature of the stimulus was detected.

*Short-range movements over each stimulus.* When the number of nose passages over the midline of the odor sectors was summed over the total time of the test, it appeared that nonfamiliar HM induced nearly 2 times more short-range head movements than did nonfamiliar FM, $t(11) = 2.94, p < .01$ (Table 4).

---

**Table 3**

Temporal Distribution of Head Scanning Movements (M ± SD) per Period of 15 s and per Trial in the Paired-Odor Test

<table>
<thead>
<tr>
<th>Experiment</th>
<th>0–15 s</th>
<th>15.1–30 s</th>
<th>30.1–45 s</th>
<th>45.1–60 s</th>
<th>0–15 s</th>
<th>15.1–30 s</th>
<th>30.1–45 s</th>
<th>45.1–60 s</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>5.92 ± 5.85</td>
<td>6.25 ± 4.58</td>
<td>6.71 ± 6.79</td>
<td>8.75 ± 5.86</td>
<td>8.00 ± 5.61</td>
<td>6.92 ± 6.47</td>
<td>7.92 ± 6.11</td>
<td>6.65 ± 4.76</td>
</tr>
<tr>
<td>3</td>
<td>10.42 ± 6.16</td>
<td>9.00 ± 4.65</td>
<td>7.83 ± 5.95</td>
<td>7.67 ± 5.37</td>
<td>10.50 ± 4.93</td>
<td>8.00 ± 5.61</td>
<td>10.92 ± 8.60</td>
<td>9.00 ± 6.88</td>
</tr>
<tr>
<td>4</td>
<td>13.30 ± 6.70</td>
<td>8.40 ± 6.55</td>
<td>11.40 ± 3.81</td>
<td>9.90 ± 6.03</td>
<td>10.00 ± 5.70</td>
<td>12.30 ± 6.72</td>
<td>9.30 ± 7.10</td>
<td>10.70 ± 5.72</td>
</tr>
</tbody>
</table>

Note. HM = human milk; FM = formula milk; f = familiar; nf = nonfamiliar; d = diluted; L = left side; R = right side.

---

Figure 2. Mean relative duration (± SEM) of head orientation in 4-day-old newborns simultaneously exposed to the odors of human milk (HM) and formula milk (FM); f = familiar; nf = nonfamiliar; d = diluted stimulus. *$p < .05$. **$p < .01$. 

---

Marlier and Schaal
In conclusion, breast-fed newborns positioned their nose longer and mouthed more actively when their nose was directed to the pad carrying nonfamiliar HM than when their nose was directed to the pad carrying nonfamiliar FM. Hence, HM is more olfactorily attractive to them than FM. The pattern of differential head orientation observed in the present test may, however, be interpreted either as reflecting a truly positive attraction to the odor of nonfamiliar HM or as resulting from a relative repulsion from the odor of nonfamiliar FM. To examine the attractiveness of nonfamiliar FM, a second group of exclusively breast-fed newborns was exposed to an absolute choice test wherein the odor of nonfamiliar FM was paired with a scentless control stimulus.

**Experiment 1a**

**Method**

Eight breast-fed infants (cf. Table 1) were simultaneously exposed to the odor of a pad impregnated with nonfamiliar FM and a pad without odorant but equalized for humidity. A previous study had shown the validity of a moistened cotton pad as a neutral (neither attractive nor repulsive) stimulus (Schaal, Marlier, et al., 1995).

**Results and Discussion**

**Head Orientation**

The mean relative duration of head orientation to the nonfamiliar FM odor (M = .430, SD = .123) differed consistently from the mean relative duration of orientation to the control stimulus (M = .238, SD = .114), F(1, 7) = 5.93, p < .05. No additional main effect of side of presentation or interaction effect between this factor and the nature of the stimulus was detected. When considered individually, significantly more newborns (7 of 8) oriented longer to the former stimulus than to the latter, $\chi^2(8) = 4.5, p < .05$.

**Oral Activation**

The infants exhibited higher oral activation when their nose was positioned over the nonfamiliar FM pad (M = .372, SD = .174) as compared with the control pad (M = .192, SD = .147), t(8) = 3.12, p < .02. Six infants expressed higher oral activation when oriented to the odor of nonfamiliar FM compared with the control pad, whereas the reverse pattern was observed in 2 infants, $\chi^2(8) = 2.0, p = .15$.

This test confirms that the odor of the nonfamiliar FM can be detected by breast-fed newborns and,

---

**Table 4**

<table>
<thead>
<tr>
<th>Odor contrasts</th>
<th>HM</th>
<th>FM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Experiment 1 nfHM vs. nfFM (breast fed)</td>
<td>12.83** ± 8.02</td>
<td>5.83 ± 5.40</td>
</tr>
<tr>
<td>Experiment 2 nfHM vs. nfFM (bottle fed)</td>
<td>9.0* ± 4.71</td>
<td>5.25 ± 3.34</td>
</tr>
<tr>
<td>Experiment 3 nfHM vs. fFM (bottle fed)</td>
<td>10.67 ± 7.31</td>
<td>7.92 ± 4.70</td>
</tr>
<tr>
<td>Experiment 4 nfHM vs. fFMD (bottle fed)</td>
<td>11.30* ± 5.69</td>
<td>7.40 ± 4.80</td>
</tr>
</tbody>
</table>

*Note. HM = human milk; FM = formula milk; f = familiar; nf = nonfamiliar; d = diluted; L = left side; R = right side. *p < .05. **p < .01.

**Oral Activation**

As relatively little mouthing was observed throughout the experiments, the side of presentation of the stimulus was not entered into the analysis. The rate of oral activation varied significantly as a function of the stimulus the infant’s nose was facing, $t(11) = 3.05, p < .01$. Namely, nonfamiliar HM induced higher oral activation than did nonfamiliar FM ($M = .221, SD = .147$ vs. $M = .160, SD = .156$; Figure 3, column A). When differential oral activation was computed for each participant, 10 of 12 participants (83.3%) exhibited higher oral activation when oriented to nonfamiliar HM than when oriented to nonfamiliar FM, $\chi^2(12) = 5.33, p < .05$. 

---

**Figure 3**

Mean relative duration (± SEM) of oral activation of 4-day-old newborns simultaneously exposed to the odors human milk (HM) and formula milk (FM); f = familiar; nf = nonfamiliar; d = diluted stimulus. *p < .05. **p < .01. ***p < .001.
more important, that this stimulus bears attractive properties. Thus, coming back to Experiment 1, it can be concluded that the positive response to the odor of nonfamiliar HM is not attributable to aversion from the paired stimulus.

Experiment 2
Although both stimulations presented in Experiment 1 were nonfamiliar, it could be argued that the odor quality of nonfamiliar HM may be more similar than FM to the milk usually ingested by breast-fed newborns. Accordingly, the differential response between nonfamiliar HM and FM noted in that experiment could be interpreted as reflecting attraction to the stimulus that is less dissimilar to the infants’ habitual milk. To assess the potential influence of odor similarity with the habitual milk on the differential orientation responses in the test choice, we repeated the same procedure with bottle-fed infants. If the relative similarity of either stimulus with the habitual milk is the most influential factor, these infants should exhibit stronger attraction to FM odor when introduced to a relative preference test opposing the scents of nonfamiliar FM and HM.

Method
Twelve exclusively bottle-fed neonates (Table 1) were tested. They were simultaneously exposed to the odor of a nonfamiliar HM and to the odor of a nonfamiliar FM (i.e., not the FM brand they had ingested since birth for which the sensory profile was sufficiently different from the familiar FM so that 4-day-old newborns could distinguish them; Marlier & Schaal, 2001).

Results and Discussion

Head Orientation

Relative duration of orientation. As shown in Figure 2, column B, the infants evinced a longer relative duration of head orientation to the pad impregnated with nonfamiliar HM ($M = .445, SD = .184$) than to the pad bearing the nonfamiliar FM ($M = .237, SD = .141$). The statistical analysis revealed a significant main effect of the odor stimulus, $F(1, 11) = 6.26, p < .03$. No additional main effect of side of presentation or interaction effect between this factor and the nature of the stimulus reached significance. When the response patterns of the 12 infants were considered, 10 of them oriented their nose longer to nonfamiliar HM than to the competing stimulus, $\chi^2(12) = 5.33, p < .03$.

Latencies to reach either stimulus. The two-way ANOVA on the latencies to orient first to either stimulus indicated a significant main effect of the odor stimulus, $F(1, 11) = 7.80, p < .02$, indicating that the newborns directed their head more rapidly toward the odor of nonfamiliar HM (right: $9 \pm 16.9$ s; left: $12.6 \pm 13.3$ s) than toward the odor of nonfamiliar FM (right: $20.8 \pm 21.5$ s; left: $30.5 \pm 23.4$ s). The side of presentation did not influence the response.

Short-range movements over each stimulus. The comparison of cephalic activity exhibited over the stimulus sectors revealed that the HM stimulus induced a higher level of search-like movements than did the FM stimulus, $t(11) = 2.26, p < .05$ (Table 4).

Oral Activation

A significant effect of the odor stimulus on oral activation, $t(11) = 2.33, p = .04$, revealed that the newborns exhibited higher activation when oriented toward the odor of nonfamiliar HM ($M = .371, SD = .178$) than when oriented to the odor of nonfamiliar FM ($M = .182, SD = .148$; Figure 3, column B). Furthermore, more insistent oral activation to HM was displayed by 9 of the 12 infants tested, $\chi^2(12) = 3.0, p = .08$.

In summary, when exposed to FM odor concurrently with HM odor, both of them being nonfamiliar, bottle-fed newborns expressed longer head orientation and higher levels of oral activation to the odor of HM. This pattern of response cannot be explained in terms of repulsion from the odor of nonfamiliar FM because that stimulus proved to be attractive in a previous absolute preference test (Marlier & Schaal, 2001).

Comparison of Breast- and Bottle-Fed Infants’ Responses

When the head orientation and oral activation responses of breast-fed infants (Experiment 1) and bottle-fed infants (Experiment 2) were compared in a two-way (Mode of Feeding × Odor Stimulus) ANOVA, no significant differences were detected, $F(1, 22) = .01$ and .42, respectively, $p > .50$ in both cases. It may thus be concluded that 4-day-old bottle-fed, as well as breast-fed, newborns demonstrate a clear preference for the odor of nonfamiliar HM when it is presented along with the odor of nonfamiliar FM. Thus, despite their contrasting experience with milk in the feeding context, both groups of infants exhibit the same preferential trend in favor of the odor of conspecific milk. The next two experi-
ments were aimed at assessing more in depth two properties that may underlie the preferential response in favor of HM: familiarity and intensity.

**Experiment 3**

The present experiment aimed to evaluate the strength of neonatal attraction to the odor of HM. It was conducted in infants bottle fed since birth in whom direct exposure to maternal breast and milk odor was indeed obviously minimal and in whom experience with a given FM brand can be precisely known. These infants were given a test pairing the odors of nonfamiliar HM and of their own FM. The direction of the infants’ response pattern to the paired stimuli cannot be predicted from existing data, and all three possible outcomes are of theoretical interest: (a) the attraction to HM odor could surpass that of FM odor, suggesting the precedence of a predisposed response over a recently learned response; (b) both stimulations may be equally attractive, indicating that a predisposed preference remains as motivationally active as a recently acquired preference; and (c) the attraction to FM odor may override that of HM odor, indicating a strong influence of the recency of learned cues.

**Method**

Twelve infants bottle fed since birth (cf. Table 1) were tested at a mean age of 92.5 h (SD = 15.8 h). They were exposed simultaneously to the odor of their familiar FM (brand Gallia 1) and to the odor of a nonfamiliar HM. Their exposure rate to FM in terms of subjective intensity (FM = .364, SD = .122) as to the pad odorized with nonfamiliar HM (M = .364, SD = .122) as to the pad odorized with familiar FM (M = .306, SD = .117; Figure 2, column C). A two-way (Odor x Side of Presentation) ANOVA evinced no main effect of the stimulus factor on the duration of head turning, F(1, 11) = 1.17, p = .30. The analysis yielded no additional main or interaction effects. Finally, the number of infants who oriented longer to the HM pad did not differ in a significant way from the number of infants orienting longer to the FM pad (8 of 12 vs. 4 of 12, respectively), \( \chi^2(12) = 1.33, p > .20 \).

**Results and Discussion**

**Head Orientation**

*Relative duration of orientation.* The infants oriented approximately as long to the pad odorized with nonfamiliar HM (M = .364, SD = .122) as to the pad odorized with familiar FM (M = .306, SD = .117; Figure 2, column C). A two-way (Odor x Side of Presentation) ANOVA evinced no main effect of the stimulus factor on the duration of head turning, F(1, 11) = 1.17, p = .30. The analysis yielded no additional main or interaction effects. Finally, the number of infants who oriented longer to the HM pad did not differ in a significant way from the number of infants orienting longer to the FM pad (8 of 12 vs. 4 of 12, respectively), \( \chi^2(12) = 1.33, p > .20 \).

**Latencies to reach either stimulus.** When the infants’ latencies of first head turn in either odor-side combinations were compared, no main nor interaction effects were revealed (HM right: 13.2 ± 13.4 s; left: 14.3 ± 23.3 s; FM right: 14.1 ± 18.5 s; left: 11.8 ± 20.4 s).

**Short-range movements over each stimulus.** The number of head-scanning movements recorded over the HM sector appeared to be slightly higher than that over the FM sector (Table 4), but this difference was only marginally significant, t(11) = 1.91, p = .08.

**Oral Activation**

Both odorants significantly affected oral activation, t(11) = 4.08, p < .002. The nonfamiliar HM odor induced a higher oral activation than did the familiar FM odor (M = .361, SD = .186 and M = .216, SD = .164, respectively; Figure 3, column C). If differential oral activation was computed for each participant, 11 of 12 participants (91.6%) exhibited a higher ratio of oral activation when oriented to the nonfamiliar HM stimulus than to the familiar FM stimulus, \( \chi^2(12) = 8.33, p = .004 \).

Bottle-fed newborns did not display differential head-turning responsiveness when concurrently exposed to nonfamiliar HM and to the FM they have ingested since birth. As the odor of familiar FM has been otherwise shown to be attractive to bottle-fed newborns when it is presented along with a control stimulus (Marlier et al., 1998b), the present result leads to the interpretation that nonfamiliar HM and familiar FM carry equally attractive odor cues. This view is, however, qualified by the direction of the oral activation index. Although the odor of HM does not elicit a more persistent head-turning response than the familiar FM odor, it is more active on oral movements.

**Experiment 4**

Experiments 2 and 3 lead to the hypothesis that HM carries attractive odor cues, the reinforcing power of which is equivalent to those of FM. An alternative interpretation may be raised, however, before this hypothesis can be fully appreciated: The differential response might as well be the result of an intensity mismatch between both stimulations. The odors of HM and FM are clearly differentiable by adult judges in terms of subjective intensity (FM > HM; Sousignan, Schaal, Marlier, & Jiang, 1997), and one cannot exclude that neonates may have the same discriminative ability. Following the logic of Schneirla’s (1965) hypothesis on intensity-based
preferences of newborn organisms, it cannot be excluded that the pattern of response seen in the previous experiments could be caused by an attraction for low-intensity relative to high-intensity stimulations. The potential involvement of the intensity factor in neonatal odor responsiveness was thus assessed in Experiment 4, in which both stimulations were equalized in subjective intensity.

Method

A sample of 10 bottle-fed infants (Table 1) was tested on average 86.4 h (SD = 15.2 h) after birth. A forerunning study was conducted with adult participants to find a FM stimulus that matches HM in subjective olfactory intensity (Meilgard, Civille, & Carr, 1991). Nonsmoker adults (n = 13; M age = 25.6, SD = 4.3 years) rated the intensity of six dilutions (100, 25, 6.25, 1.56, 0.39, and 0.097%) of the habitual FM brand (Gallia 1) as well as of the pure solvent (distilled water). The ratings were obtained for each dilution and the solvent by marking a cross on a 15-cm line scale ranging from 0 (not strong at all) to 15 (very strong). The samples (15 ml) were presented in random order in 60-ml opaque glass jars. Each participant rated the intensity of each sample using a reference sample of HM (15 ml; obtained by pooling four samples of HM to reduce individual variability). The intensity value of HM, arbitrarily set at 7.5 cm, was indicated in the middle of the scale. The judges could sniff the HM reference as often as they needed throughout the session. To limit olfactory adaptation, an intertrial interval of 1 min was paced by the experimenter between each sample rating. This approach involved the selection of a dilution of FM with a subjective intensity that matched that of HM. This dilution step (1.56% v/v) scored on average 7.6 cm (SD = 3.1 cm) on the intensity scale. Accordingly, the newborns were exposed to a nonfamiliar HM and the 1.56% dilution step of the FM they were used to consuming since birth.

Results and Discussion

Head Orientation

Relative duration of orientation. A two-way (Odor × Side of Presentation) ANOVA revealed a main effect of the odor stimulus on the duration of head orientation, F(1, 9) = 15.28, p < .004, indicating that the newborns oriented longer to the odor of the nonfamiliar HM than to the odor of the diluted FM (M = .422, SD = .090 and M = .221, SD = .086, respectively; cf. Figure 2, column D). No additional main or interaction effects reached significance. Seven newborns oriented their nose longer to the odor of HM than to the odor of diluted FM, whereas 3 newborns exhibited the reverse pattern, χ²(10) = 1.60, p = .20.

Latencies to reach either stimulus. Participants evinced significantly shorter average latencies to first position their nose over the nonfamiliar HM sector (right: 4 ± 6.6 s; left: 6.9 ± 11.3 s) than over the diluted familiar FM (right: 5.9 ± 7 s; left 17.8 ± 19s), F(1, 11) = 7.57, p < .023. Neither the presentation side nor its interaction with the odor factor affected the latency of first head orientation.

Short-range movements over each stimulus. Infants simultaneously exposed to nonfamiliar HM odor and the diluted familiar FM odor appeared to be more activated by the former stimulus in terms of amount of search-like movements over the corresponding stimulus sector, t(9) = 2.45, p < .05 (Table 4).

Oral Activation

A significant effect of the odor stimulus on oral activation, t(9) = 2.71, p = .02, further indicated that these newborns exhibited increased oral activation when oriented to the nonfamiliar HM than when oriented to the diluted familiar FM (M = .368, SD = .133 and M = .233, SD = .097, respectively; cf. Figure 3, column D). A more persistent oral activation to HM was displayed by 8 of the 10 infants tested, χ²(10) = 3.6, p = .058.

In sum, bottle-fed newborns expressed longer head orientation and higher oral activation to the nonfamiliar HM when concurrently exposed to the iso-intensive odors of HM and FM. Thus, the lessening of the odor intensity of the familiar FM appears to restore fully the relative attraction of nonfamiliar HM odor noted in Experiments 2 and 3.

Experiment 4a

A last experiment was undertaken to ascertain whether the odor of the diluted FM is effectively detected by, and remains attractive to, the newborns in the previous experiment. Dilution may indeed reduce stimulus intensity to levels below the infants’ threshold or alter the stimulus quality so that newborns are no longer able to recognize it. In both cases, infants would cease to value it positively and would change their responsiveness

Method

Exclusively bottle-fed infants (Table 1) were studied in a choice test consisting of the odor of their
familiar FM (brand Gallia 1) diluted in distilled water (concentration: 1.56% v/v) versus a scentless pad wetted with distilled water.

Results and Discussion

Head Orientation

A two-way ANOVA yielded a significant main effect of the odor stimulus, $F(1, 12) = 6.04, p = .03$, indicating a longer relative duration of head orientation to the odor of the diluted familiar FM pad than to the control stimulus ($M = .392, SD = .176$ and $M = .215, SD = .115$, respectively). No additional main effect of side of presentation or interaction effect between this factor and the nature of the stimulus was found. When individual responses were considered, 10 of the 13 infants oriented longer to the diluted FM odor than to the control stimulus, $\chi^2(13) = 3.77, p = .052$.

Oral Activation

The diluted familiar FM induced higher oral activation than did the control stimulus ($M = .407, SD = .246$ and $M = .195, SD = .210$, respectively), $t(12) = 5.60, p < .001$. Twelve of 13 infants expressed higher oral activation when facing the odor of diluted familiar FM as compared with the control stimulus, $\chi^2(13) = 9.31, p < .003$.

This last experiment confirmed that, even after considerable dilution (1.56%), bottle-fed newborns can detect and remain attracted to the odor of their habitual FM. Thus, the absolute attractive properties noted in a previous study with plain FM (Marlier et al., 1998b) is apparently not altered by the attenuation of the stimulus concentration. One may then conclude that the differential response noted in Experiment 4 is based on a preference for the odor of the unfamiliar HM compared with the odor of the diluted familiar FM and not by either the infants’ inability to detect or their repulsion from the latter.

General Discussion

The present series of experiments was designed (a) to examine whether the odor of HM was attractive to human infants and (b) to evaluate the degree of dependence of this attractiveness on direct exposure with HM after birth. Both of these objectives were put to trial by presenting newborns with the odor of HM paired with the odor of a FM of bovine origin, both substrates being unfamiliar to them. The results were that breast-fed (Experiment 1) as well as bottle-fed infants (Experiment 2) evince more persistent head-orientation and mouthing responses to HM odor. This pattern of relative preference was not surprising for breast feeders, who were expected to direct their appetitive responses toward the odor stimulus that resembles most the one they were fed (i.e., maternal milk). But it was unanticipated that bottle feeders would also orient and mouth longer to the odor of HM, the stimulus that matched least their habitual FM. This latter result points to specific properties of attraction linked with HM.

Two conclusions may be reasonably drawn from these experiments. First, HM (at least when collected 3–4 days postpartum) carries volatile compounds that induce positive behavioral activity in human infants. As extreme care was taken to sample the milk while it pearled at the tip of the nipples, it was devoid of any of the other exocrine secretions emitted from areolar glands (e.g., Schaal & Porter, 1991). Thus, odorants contained in milk alone may account for the behavioral effect engendered by the whole breast odor of lactating females reported in previous studies (Macfarlane, 1975; Makin & Porter, 1989; Russell, 1976; Schaal et al., 1980). Second, the odor of HM elicited positive orientation and enhanced mouthing in the infants regardless of their postnatal experience with it (i.e., whether they were breast or bottle fed). Specifically, the response pattern of the infants in our sample who were never exposed to lactating breasts or to HM (Experiment 2) argues against postnatal experience dependency of the attractive power of HM odor.

The present study also aimed to appraise the relative reinforcing potency of the odor of HM. To do so, bottle-fed infants were given the simultaneous choice between the odors of a novel stimulus (HM) and of a stimulus known to be positively reinforced before the test (their familiar FM; Experiment 3). These infants evinced nondifferentiated duration of head orientation to either odor stimulation, a result that may be interpreted in terms of motivational equivalence between the odor of HM and the odor of the FM associated with 20 to 25 episodes of satiation. Nevertheless, when facing either stimulus, the infants still expressed differential mouthing movements, indicating that the contrast in appetite for one odorant over the other was persistent regardless of the familiarity status of the FM (Experiments 2–4). This result reinforces the notion that HM bears special behavioral properties for newborns and that the most recently acquired stimulus (i.e., FM) is not necessarily the most attractive. However, regarding the nature of the paired stimuli, a confound must be stressed. HM was freshly obtained without further transformations.
other than possible cooling or freezing, whereas FM was subjected to numerous severe treatments (skimming, multiple additions, sterilization) so that its native properties may not be all conserved. Thus, although of heterospecific origin, FM was not the natural version of cow's milk. To fully assess species specificity in the neonatal response to HM, future experiments should test it against freshly ejected bovine milk or milk from any other species.

Experiments 4 and 4a aimed to rule out the eventual impact of an intensity confound in the test opposing the odors of HM and of FM. Because HM is less olfactorily intense than FM (Jiang, Soussignan, Marlier, & Schaal, 2004), FM was diluted to reach olfactory iso-intensity with HM while remaining detectable and attractive to infants (Experiment 4a). Despite this precaution, the results confirmed a higher level of attraction of HM over FM.

This study gives credit to the notion that HM contains volatile substances that have a positive meaning for human newborns, which is not conditional on previous postnatal exposure to them. Why do newborns prefer a conspecific stimulus to which they were never directly exposed over a heterospecific stimulus received repeatedly in rewarding conditions? This issue is reminiscent of parallel investigations performed on newly emerged organisms of other species that were put in a choice situation between conspecific social stimuli and cues of arbitrary or heterospecific nature. For example, when newly hatched chicks that were preexposed for 24 h to either a red box (Chicks B) or a stuffed hen (Chicks H) were given the simultaneous choice between the red box and the stuffed hen, Chicks H displayed the expected orientation to the stuffed hen whereas Chicks B evinced equivalent attraction to the box to which they were exposed and to the stuffed hen that they never encountered before in any form (Johnson, Bolhuis, & Horn, 1985). An additional group of freshly hatched chicks, naive to both box and stuffed hen, also manifested a clear preference for the hen when they were given the choice (Johnson et al., 1985). Developing chick responses to visual objects are hence organized by the joint operation of a learned preference and of an inherent tendency to approach a given visual shape without prior specific experience with it. Mallard ducklings present another classical example of nonrandom responsiveness in the auditory domain. Regardless of posthatching auditory experience, emerging ducklings direct themselves toward a loudspeaker emitting the typical maternal vocalization (Gottlieb, 1971). If they receive ex ovo exposure to the maternal vocalization of another species, they subsequently approach the heterospecific and conspecific vocalizations equivalently (Gottlieb, 1981). Thus, posthatching auditory experience does not interfere with the preexistent bias to the conspecific maternal call. But, in contrast with the chick’s visual preference, ducklings depend on prehatching auditory experience: The embryo has to hear its own vocalizations to develop the species-typical behavior (Gottlieb, 1971). Both of these examples underline that the appearance right after emergence of nonrandomly oriented behaviors can be multiply determined. They identify two developmental pathways leading to the expression of species-specific behavior, one relying on neurocognitive abilities that go uninfluenced by any obvious antecedent sensory impact and another array of abilities molded by direct exposure to species-specific cues in the (embryonic or neonatal) environment.

The data from the present study suggest that, much like avian offspring, mammalian neonates may have evolved biased responses to certain species-specific stimulus of vital significance, namely, milk. Human newborns are not an exception among mammals in their predisposed olfactory attraction to conspecific milk. For example, newly born rabbits presented with milks of murine, ovine, bovine, equine, or human origins remain behaviorally inert, whereas they are extraordinarily aroused by rabbit milk (Keil, von Stralendorff, & Hudson, 1990; Schaal et al., 2003). Second, the preferential bias in favor of conspecific milk odor is not hindered by the competitive learning of odor stimuli that recruit the same effector systems. In the human infant, Experiments 3 to 4 of the present study show that the repeated exposure to heterospecific milk does not alter the positive response to HM. Moreover, the association of an artificial odorant with breast feeding does not obviate subsequent responsiveness to HM in a test opposing it with this novel odorant (Delaunay-El Allam, Marlier, & Schaal, 2002). Newborn rabbits behave much alike in that pups bottle fed with a bovine-milk-based formula do not evince any alteration in their typical response to conspecific milk (Coureaud, Schaal, Langlois, & Perrier, 2001). Such a powerful bias in favor of conspecific milk odor may be explained, as in the previous avian cases, either by learning in the preceding developmental niche or by the behavioral actualization of a genetically programmed stimulus—response coupling.

Regarding the possibility that neonatal attractiveness to conspecific milk odor might be conditional on antecedent learning, strong evidence is at hand that mammalian fetuses can acquire and retain olfactory information (Hepper, 1996; Schaal, 1988; Schaal, Orgeur & Rognon, 1995). Odor cues learned
in utero remain useful to the neonate, as the same odors are partially externalized into the postnatal environment as components of maternal secretions such as colostrum, milk, or other skin exudates (Schaal, in press). This chemosensory overlap between amniotic and lacteal fluids is substantiated by the neonates’ equal distribution of head turning between the odors of their own amniotic fluid and the mother’s colostrum (Marlier et al., 1998a). In contrast, in a similar test that opposed the odors of their own amniotic fluid and that of their familiar FM, bottle-fed newborns oriented more to the former (Marlier et al., 1998b). Thus, one possibility explaining the development of a neonatal bias in favor of conspecific milk may be based on odor information acquired while in the amniotic fluid.

The second way to render milk attractive would be for females to transmit to it a chemosensory factor that releases unconditional responsiveness in the offspring, that is, responses that do not depend on prior sensory experience. One such prespecified signal was recently described in the rabbit newborn for which the positive response to rabbit milk is based on a single molecule: the mammary pheromone (Schaal et al., 2003), emitted de novo in milk (Moncombe et al., in press) and does not require association with suckling, ingestion of milk, or contact with the mother. Furthermore, chemical analyses of amniotic fluid and blood from lactating females having failed to detect it (Schaal et al., 2003), its behavioral activity does not appear to derive from direct prenatal experience. Accordingly, the current data indicate that newborn rabbits are endowed with specialized odor-guided responses that may preexist direct sensory exposure. So far it remains uncertain whether similar unconditionally reactogenic odors are produced in other mammals. Converging questions about the existence of odorants from lactating breast that release unconditionally appetitive responses in human newborns have been raised repeatedly (Russell, 1976; Schaal, 1988) but never pursued adequately. The present study is a first contribution to the assessment of such a possibility.

In conclusion, human infants respond by increased head orientation and mouthing activation to the odor of HM. The behavioral activity carried in conspecific milk is equal to or greater than that of an heterospecific FM, even when the latter has become reinforced through recurrent association with satiety. Furthermore, the behavioral potency of HM odor is not markedly mitigated by the familiarity or intensity features of the competing stimulus presented in the present two-choice paradigm. The attractive or appetitive activity of HM odor may thus seemingly arise in the absence of specific information from the postnatal environment and remains resistant to changes in the environment because of novel acquisitions. Its development may presently be explained either by fetal odor memory associated with a relative continuity between amniotic and lacteal scents, or by the operation of an experience-independent odor-guided behavior based on a specialized signal.

References


