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Olfactory Conditioning Facilitates Diet Transition in Human Infants

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ABSTRACT: We evaluated whether Pavlovian conditioning methods could be used to increase the ingestion of non-preferred solutions by formula-fed human infants. In baseline measures, 5–7 month old infants sucked less frequently and consumed less water than regular formula. During a 3-day olfactory conditioning period, parents placed a small scented disk, the conditioned stimulus, on the rim of their infants' formula bottle at every feeding. Following this training, infants' responses to water were tested when their water bottles had a disk scented with the training odor, a novel odor, or no odor. Infants tested with the training odor sucked more frequently and consumed significantly more water than they had at baseline. Infants tested with no odor or a novel odor consumed water at or below baseline levels. These data demonstrate that olfactory conditioning can be used to enhance ingestion in infants and suggest that such methods may be useful for infants experiencing difficulty when making transitions from one diet to another. © 2000 John Wiley & Sons, Inc. *Dev Psychobiol* 37: 144–152, 2000

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Feeding difficulties are frequently experienced by human infants who, for medical reasons such as metabolic disorder or allergy, must make transitions to new and often unpleasant-tasting formulas (Vandenplas, Hauser, Van den Borre, Sacre, & Dab, 1992). We evaluated whether sucking might be enhanced, and thus diet transitions facilitated, by applying olfactory conditioning techniques similar to those that have been shown to influence ingestion in developing animals. Such an approach would take advantage of the role of olfactory stimuli in influencing the flavor

and perception of foods (Rozin, 1982; Poothullil, 1995) and in guiding and influencing early ingestive behavior (Rosenblatt, 1971; Alberts, 1984; Schaal, 1988; Schaal, Orgeur, & Porter, 1994).

Human infants are very responsive to olfactory stimuli. Within the first 2 weeks of life, breast-fed infants discriminate pads scented with their mother's odor over those from another mother (Macfarlane, 1975; Cernoch & Porter, 1985; Porter, Makin, Davis, & Christensen, 1992), and 6-week-old breast-fed infants respond to the scent of their own mother's breast pad with a selective sucking response (Russell, 1976). Older infants modify their sucking when odors in the breast milk reflect changes in their mother's diet (Mennella & Beauchamp, 1991, 1993; Mennella, 1995). Bottle-fed infants are not exposed to as wide a variety of olfactory cues in their diet as are breast-fed infants, yet they are still sensitive to the olfactory characteristics of their formula and change their sucking pattern in response to novel odors (Mennella & Beauchamp, 1996a).

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Even in very young infants, odors are effective stimuli for conditioning. One-day-old infants who receive odor stimuli paired with a form of gentle stroking turn their head towards that odor on the next day (Sullivan et al., 1991). One-day-old rat pups learn to probe into soft paddles over their heads in order to receive milk infusions into their mouths, and can learn to distinguish which paddle provides a milk infusion on the basis of an odor on the paddle (Johanson & Hall, 1979). When odor conditioned stimuli are paired with milk infusions into their mouths, rat pups subsequently not only spend more time in contact with the odors (Johanson & Hall, 1982), but also mouth, probe and become behaviorally active when presented with the odor alone (Johanson, Hall, & Polefrone, 1984; Terry & Johanson, 1994). Furthermore, in infant mammals, odors that have been paired with milk are capable of influencing the ingestion of non-preferred solutions (Johanson & Terry, 1988).

Here we evaluated whether ingestion of a non-preferred solution could be enhanced in human infants using techniques similar to those employed with animals. Our studies were also influenced by the finding that, in adult rats, stimuli or signals paired with feeding can themselves come to induce ingestion (Weingarten, 1983, 1984). In the present study, we used a scented disk (Flavor-Dots [Patent Approved]) with which a food-related odor can be added to the rim of an infant's bottle without adding anything to the formula itself. This technique allows an odor to be associated with sucking the familiar and safe formula from the bottle. During the training interval, parents who were bottle-feeding their infants with formula placed the scented disk on the rim of their infant's bottles at every feeding for 3 days. We then transferred that odor to a new, non-preferred fluid to determine if the trained odor could facilitate the introduction of that fluid. In this study, we evaluated the effect of olfactory conditioning on the intake of plain tap water. Water has no taste or nutrient value per se and it is normally not avidly consumed by infants younger than 6 months of age (Desor, Maller, & Turner, 1973; Maller & Desor, 1974; Desor, Maller, & Andrews, 1975). It thus presents a good test of the conditioning effect.

METHOD

Participants

Participants were recruited from the birth records of Wake and Durham Counties, NC, USA. The data presented are from 24 healthy full-term infants (16 female, 6 male) aged 131–220 days (average

age = 174 days, SD = 23.5). There were 19 Caucasian and four African–American infants, and one infant of Caucasian and Asian heritage. Family incomes ranged from less than \$10,000 to greater than \$50,000 per annum. Infants had not changed formulas within the month prior to the start of the study, and received the majority of their caloric intake from the following standard infant formulas: Similac ($n = 8$), Enfamil ($n = 4$), Lacto-free ($n = 2$), Prosobee ($n = 1$), Nutramagen ($n = 1$), Alimentum ($n = 1$), unknown ($n = 7$). All but two infants had been introduced to infant cereal, fruits, vegetables and fruit juice. None had had experience with drinking water from a bottle prior to participating in this study. Six other infants were excluded because their families used a test solution other than water or the infants developed transient illnesses that could interfere with their olfactory sensitivity. Families were paid for their participation in the study.

Flavor-Dots. The scented disks (Flavor-Dots) that were used for the study are small (approximately 60 mm) disks of absorbent material, impregnated with dry, nontoxic releasable odors from standard food flavor additives. The disks are backed with adhesive and provided to the parents in arrays on glossy release sheets. Each dot is peeled off and applied to the rim of an infant's bottle for the duration of the feeding. When a disk is dampened, the odor begins to be vaporized from the disk, providing a weak but distinctive odor in the region of the disk for 20–40 min, so that an infant smells the odor while ingesting his or her regular formula.

Training. Following a baseline measure of intake and sucking in response to plain, unscented water, and unscented formula, parents began pairing the training odor with their infant's regular formula during every feeding for 3 days. Parents were asked to place the disk scented with the training odor on the rim of every formula bottle and slightly dampen it so that their infant could smell the odor while ingesting formula. The odor was not added to formula itself so that an infant did not actually ingest any of the extracts used to manufacture the scented disks. Parents recorded the date and time of each exposure to the training odor on a standardized sheet. In this study, half of the subjects were trained with vanilla-scented disks and the other half trained with strawberry-scented disks.

Testing. On each of three test days, two researchers visited the infant's home to observe the feeding session. Each family's visits were scheduled for the same time of day, approximately 2 hr after a regular

feeding. Parents provided formula and water warmed to formula temperature, and infants were fed with their own bottles by their regular caretakers. In order to conduct multiple trials of the initial acceptance of a solution without a confounding ceiling effect due to satiation, each infant was given three trials of 120 s each. To determine intake, one observer weighed the test bottle prior to and after each trial, and, on the test days, placed the test disks on the bottles in an order unknown to the parent and to the second observer. The second observer recorded the number of times the infant sucked from each bottle by visually scoring cheek and chin movement. The total number and patterns of sucking behavior were recorded on a palmtop computer programmed as an event recorder to log the timing of each suck during the 120 s long trial. Observers terminated a trial before 120 s if the infant refused the bottle three times by pushing the bottle away, crying, or otherwise indicating distress.

During the baseline day, details of the project were explained to the parents, and the consent forms were signed. Then, the parents were asked to feed their infant in three trials. On the first trial, infants were presented with a bottle which contained tap water warmed to the same temperature as their typical formula. In the second and third trials, infants were presented with their regular formula, without (Trial 2) and then with (Trial 3) the disk scented with the training odor.

The effects of conditioning were tested after 3 days of pairing the scented disks with the infants' regular formula. On the test day, infants were given three trials: disk scented with the training odor, disk scented with a novel odor, and an unscented disk. The trials were separated by the time it took to weigh the test bottle and place the new scented disk on the bottle. The order of presentation of the test stimuli was counterbalanced across infants. For infants trained with vanilla, strawberry was the novel odor, and for infants trained with strawberry, vanilla was the novel odor.

To assess the extended effects of training, infants were given a retention test 6 days after the first test. During the interval between the conditioning test and the retention test, infants were not exposed to water or either of the odors. During the retention test, stimuli were presented in the same order that they had been presented initially.

RESULTS

Baseline

Baseline sucking and intake data indicated that water was not a preferred substance. Infants consumed less

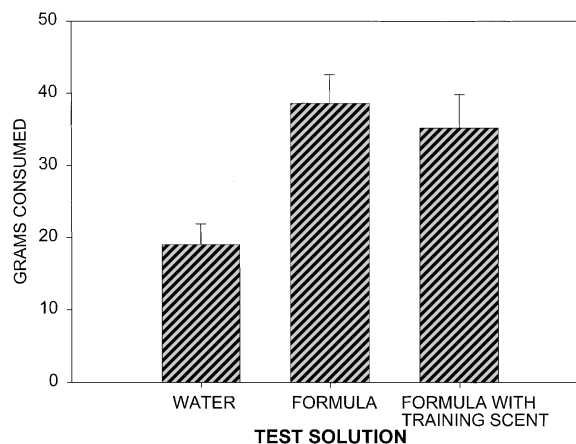


FIGURE 1 Baseline intake of water, formula, and formula with the training scent. In 2 min trials, 5–7-month-old infants ($n=24$) consumed less water than formula. There were no differences in intake between the formula trials without and with the training scents (vanilla or strawberry).

water than they did formula (see Figure 1. Intake: $F(2, 65) = 7.11, p < .01$). Infants sucked from the bottles containing water less frequently than they did bottles of formula (Figure 2. Test Solutions: $F(2, 414) = 96.6, p < .01$). Infants sucked more frequently at the beginning of each 2 min trial than at the end (Time: $F(5, 414) = 6.15, p < .01$) but there was no interaction between the test solutions and time ($F(10, 414) = 0.8, p = n.s.$). The addition of an odor to an infant's regular formula did not significantly alter

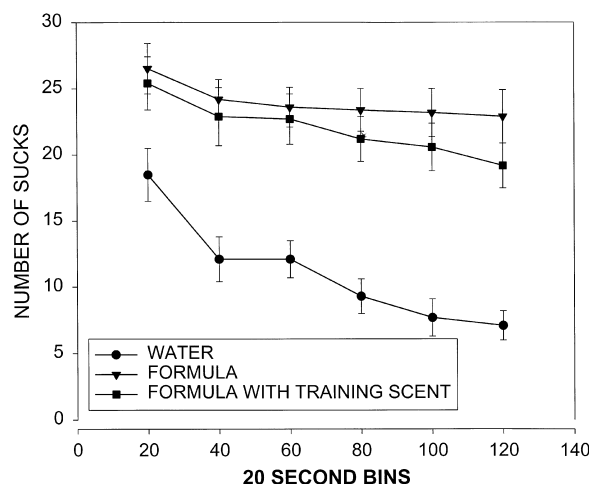


FIGURE 2 Baseline sucking responses to water, formula and formula with the training scent. Infants ($n=24$) sucked less frequently when their bottles contained water than when they contained their regular formula. Adding the training scent to the rim of an infant's bottle did not change the rate or amount of sucking from their formula bottle.

either the intake of formula, or the pattern of sucking. There was no difference in the initial response to the odor of vanilla vs. strawberry, suggesting that infants did not have an intrinsic preference of one scent over the other (Intake: $F(1, 17) = 0.02, p = \text{n.s.}$; Number of Sucks: $F(1, 21) = 0.37, p = \text{n.s.}$).

Conditioning Test

During the interval between the collection of baseline data and the conditioning test, the infants received an average of $14 \pm .74$ pairings of the scented disks their regular formula feedings. There were no differences among test groups in the number of pairings the infants received.

Trial 1: Intake. On their first trial, infants received one of three disks: the training odor, the novel odor, or no odor ($n = 8$ per group). Infants who were tested first with the training odor consumed significantly more than they had during baseline (see Figure 3. $F(2, 21) = 5.31, p < .01$). Infants given the unscented disk on their first trial, and infants tested with the novel odor consumed slightly, but not significantly, less water than they had on their first experience with water. Seven out of eight infants tested with the training odor consumed more than they had at baseline, while most of the infants tested with the unscented ($n = 7$) or untrained ($n = 6$) disks consumed the same or less water than they had during baseline.

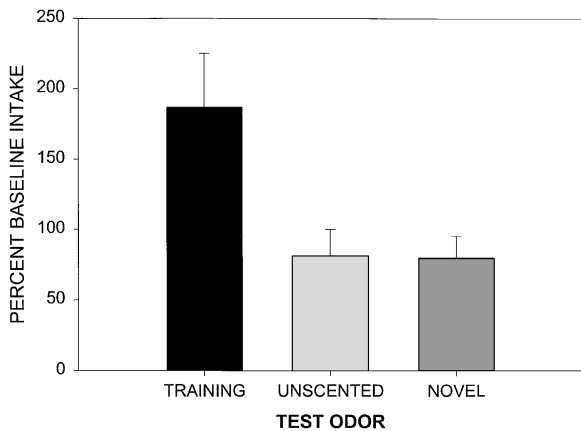


FIGURE 3 Intake of water on the first trial after conditioning. Infants ($n = 8$ per group) tested with the training odor on the first trial consumed more than they had during the baseline test, while infants tested with an unscented disk or a novel odor consumed slightly less than they had at baseline.

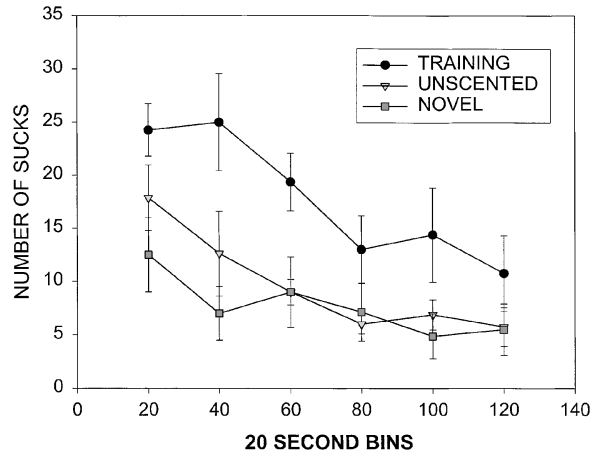


FIGURE 4 Pattern of sucking during the first trial after conditioning. Infants ($n = 8$ per group) tested with the training odor on the first trial after conditioning sucked more frequently than did infants tested with an unscented disk or a novel odor.

Trial 1: Sucking. The pattern of sucking over the 2-min test was also significantly different in infants who received the training odor on their first trial compared to that of subjects who were tested with an unscented disk or the novel odor. Infants tested with the training odor sucked more frequently throughout the 2-min test than did infants in the other two groups (see Figure 4. $F(2, 126) = 19.80, p < .01$). All infants sucked more frequently at the beginning of the 2-min test than at the end ($F(5, 126) = 6.20, p < .01$), although there was no interaction between test odor and time ($F(10, 126) = 0.71, p = \text{n.s.}$). The infants' initial sucking rates with the training odor were similar to their sucking with their own formulas during baseline.

Trial 2: Intake. After the first trial, the three groups were divided so that half of the subjects in each group were tested in one of the remaining odor conditions, and the other half were tested in the other ($n = 4$ per group). Both the test condition in the first trial and the infants' training appeared to influence the intake of water on Trial 2 (see Figure 5, $F(5, 12) = 17.52, p < .01$). The effects of test order and training were most pronounced if the training odor had been presented in the first trial. Infants who received the unscented disk after the training odor consumed at least twice their baseline amount, even though the water on the second trial was unscented. In contrast, the subjects who received the novel odor following the training odor consumed less than they had during baseline.

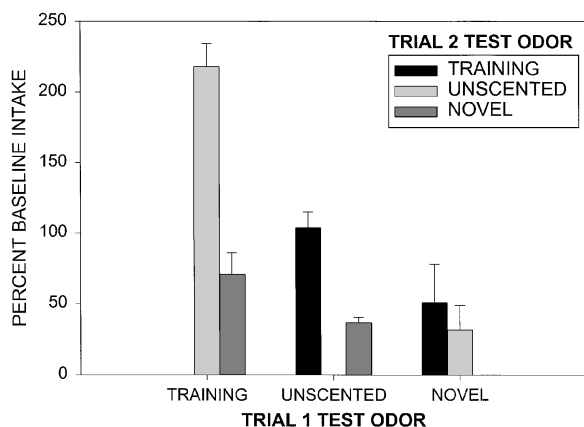


FIGURE 5 Intake of water on the second trial after conditioning. The odor the infants had received in Trial 1 and the olfactory conditioning influenced intake on the second trial after conditioning. Those infants tested with the training odor on the first trial consumed more water than they had at baseline when tested with an unscented disk, but less than baseline when tested with the novel odor. Infants tested with the unscented disk on the first trial consumed water at baseline levels when tested with the training odor, but consumed less than 50% of their baseline levels when it was scented with the novel odor. Infants tested with the novel odor on the first trial consumed 50% or less than baseline levels, regardless of the test odor on the second trial ($n = 4$ per group).

Infants who were tested with the unscented disk in Trial 1 followed by the training odor in Trial 2 consumed about what they had during the baseline. However, infants who received the novel odor after the unscented disk consumed approximately one third the amount of water that they had consumed during baseline. Thus, infants were still willing and able to discriminate between the odors if the initial exposure to water was unscented or neutral. In contrast, infants who were tested with the novel odor on Trial 1 were not willing to consume water during Trial 2, regardless of whether the second trial was with the training odor or the unscented disk.

Trial 2: Sucking. The total number of sucks during the 2-min trial is presented in Figure 6. The general pattern of sucking in Trial 2 follows that of intake, in that the infants who had received the training odor on Trial 1 followed by the unscented disk in Trial 2 sucked most frequently and for the longest time. All other groups sucked infrequently, and had virtually ceased sucking by the end of the second 2-min trial (Test Odor: $F(5, 36) = 4.93$, $p < .01$; Time: $F(5, 36) = 11.46$, $p < .01$; Odor by Time Interaction: $F(5, 36) = 0.86$, $p = n.s.$).

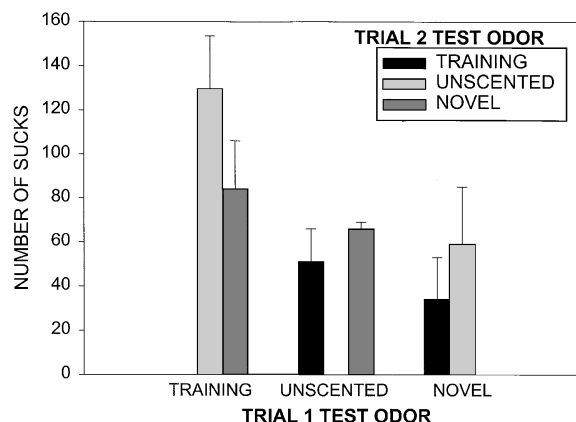


FIGURE 6 Sucking response to water on the second trial after conditioning. When tested with the unscented disk, infants who had received the training scent on the first trial sucked more frequently and longer than did all the other groups ($n = 3-4$ per group).

Trial 3: Intake and Sucking. In Trial 3, infants were offered the remaining odor that they had not received in Trials 1 and 2. By the third trial, each infant had already had two 2-min exposures to water, at least one of which was with an unfamiliar scent or no-scent. Most had already refused to continue sucking from the water bottle by the end of the second trial. During the third trial, both intake and sucking were greatly reduced in all groups. With the large variance and small number of subjects ($n = 3-4$ per group), there was no statistical difference among test groups in intake ($F(5, 16) = 0.61$, $p = n.s.$). All infants, even those who had started out testing the solution, were unwilling to continue sucking beyond 60 s into the test (Test Odor: $F(5, 108) = 3.04$, $p < .05$; Time: $F(5, 108) = 5.6$, $p < .01$; Test Odor \times Time: $F(10, 108)$, $p = n.s.$).

Retention Test

Because of a generally low intake and rate of sucking during the retention test, we present the data from the first trial only. During the retention test, water intake was more than 50% below the baseline level, and there was no difference in intake among infants tested with the training odor, the novel odor, or the unscented disk ($F(2, 15) = 0.18$, $p = n.s.$).

The total number of sucks for each group was also less than that observed in the initial tests. The pattern of sucking, however, suggests that some aspect of the prior pairing of an odor with formula or with water may have been retained over the interval. Infants tested with the training odor sucked more frequently than did those tested with the novel odor or unscented

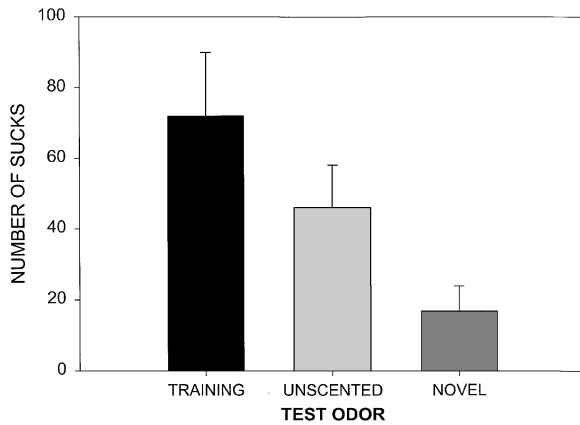


FIGURE 7 Sucking response after a 6-day retention interval. Infants tested with the training odor sucked more frequently than did infants tested with the unscented disk, while infants tested with the novel odor that had been paired with water on the first trial after conditioning avoided sucking from the water bottle ($n = 6$ per group).

disk (see Figure 7; Test Odor: $F(2, 24) = 7.89$, $p < .01$). The infants who had had a novel odor paired with water on the first trial 6 days earlier did not suck from the bottle scented with that novel odor throughout the entire 2-min retention test.

DISCUSSION

Basic Conditioning

We utilized scented disks to repeatedly pair a food-related odor with infants' regular formula while they were ingesting it, then tested whether that odor would increase the acceptance of a non-preferred novel solution. Infants did not adjust their intake or sucking response when a novel odor was added to their familiar formula. However, on the first trial after conditioning, infants aged 5–7 months consumed 187% of their baseline intake of water if their bottles were scented with the training odor, compared with only 80% of baseline intake if their bottles were either unscented or scented with the novel odor. Infants tested with disks scented with the training odor also sucked more frequently and longer than did infants tested with unscented disks or disks scented with the novel odor. Their increased intake and sucking response was dependent on the olfactory conditioning training, and not an intrinsic preference for vanilla or strawberry scents, in that there were no differences in the baseline response to these two odors. In addition, infants trained with vanilla preferred vanilla over strawberry, and infants trained with strawberry preferred strawberry over vanilla.

Test Sequencing Effects

During their initial tests, infants were exposed to three different trials, with the order of presentation counter-balanced across groups. On the second trial, it became very apparent that the infants were paying attention to the stimulus properties of the odors and the water itself. Infants tested with the disk scented with the training odor on the first trial responded differently to water on the second trial, dependent upon the subsequent scent of the disk. If the water on the second trial was unscented, the infants sustained sucking over the 2-min trial, and consumed more water on the second trial than they had during baseline. Thus, in the short term, pairing the conditioned odor with water resulted in a positive response to water even when the trained odor was no longer available. On the other hand, if the water was scented with a novel odor during the second trial, the infants sucked less after the first minute, and consumed less water than they had during baseline, perhaps because the distinctly different odor called their attention to the water.

Infants who had been tested with an unscented disk in the first trial followed a similar pattern, in that they consumed water at baseline levels when it was scented with the training odor, but took only 40% of their baseline consumption if the test odor was novel. Finally, infants who had had the novel odor paired with water on the first trial avoided sucking and consuming water on all subsequent trials, perhaps having been quickly signaled that the test fluid was not formula. These results may be examples of flavor-flavor conditioning (see Capaldi, 1996, for review), in which a positive affective tone of the conditioned odor enhanced the consumption of water, while the water itself bestowed a negative affective tone to what had been a novel odor.

Retention of Conditioning

Despite the strong effect of odor conditioning on the initial acceptance of a non-preferred solution, we found mixed indications that infants formed a long lasting memory or preference with the training. Retention tests carried out 6 days after the initial tests reveal that water consumption was very low in all infants, with no difference among test odor conditions. Remember, however, that retention tests occurred after all infants had been tested with three different odor conditions paired with water, and no subsequent pairing of the training odor with their regular formula. In contrast to measures of consumption, patterns of sucking suggest that something about the previous training or test conditions may be retained over the

longer interval. Infants tested with the training odor sucked more frequently and longer than did infants tested with no odor.

The more surprising result was that, when tested after a 6-day retention interval, infants who had a single trial of a novel odor paired with water on their very first test trial—the test condition that produced the least ingestion—avoided sucking when their bottles were again scented with that odor. One interpretation is that the infants acquired a slight aversion to the novel odor after it was paired with water, presumably because of the unpleasant taste characteristics of the water, and were using the olfactory cues to avoid further consumption of that solution. In 5–7-month-old infants, water was never preferred over formula and repeated exposure to plain water on baseline and test days did not enhance its acceptance. Furthermore, the pairing of the novel odor with water resulted in a greater suppression of sucking and intake than did exposure to water alone. Studies of conditioned flavor aversion in adult (Garcia, Hankins, & Rusiniak, 1974; Rusiniak et al., 1979) and infant (Gregg, Kittrell, Domjam, & Amsel, 1978; Coyle, 1980; Rusiniak, Garcia, Palmerino, & Cabral, 1983) animals reveal that it is a very robust phenomenon, acquired in one trial, and retained over long intervals when combined with olfactory cues. A number of studies have also shown flavor-flavor learning in which the negative affective tone of one of the flavors transfers to the other flavor (Lavin, 1976; Fanselow and Birk, 1982). The present data suggest that it may be especially important for the first experience with a novel olfactory stimulus to be positive since the avoidance which results from a single pairing of a novel odor with a non-preferred solution might have long lasting consequences. The novel odor stimulus seems to exacerbate the unpleasant experience of the non-preferred solution.

What is Being Conditioned?

When an odor is paired with a familiar and preferred formula, are infants acquiring a conditioned preference for the odor, with the positive affective tone of the odor then enhancing ingestion, or is the increased intake with the training odor a result of a classically conditioned motor response? Studies with developing mammals suggest both mechanisms may be at work. When odor stimuli are paired with milk infusions into the mouths of rat pups, pups develop both conditioned preferences for the odor (Johanson & Hall, 1982) and conditioned ingestive responding (Johanson et al., 1984). Here, support for a conditioned preference or positive flavor-flavor conditioning comes from Trial 2,

in which the infants who had received the training odor during Trial 1 continue to suck and consume unscented water during Trial 2. As well, the presentation of the training odor may elicit a sucking response, and the increased intake is a consequence of the conditioned sucking behavior. The retention over a 6-day interval of an enhanced sucking response to the training odor argues for a conditioned motor response. Anecdotal evidence from the field support the idea that the pairing of an odor with an infant's formula bottle is conditioning an oral response to the odor. One child, whose test data were not included for medical reasons, would not take any liquid from a bottle, even regular formula, in the presence of the observers. When presented with the unscented and novel odor test disks, the infant rejected the water bottle and repeatedly threw it to the floor. When presented with the training odor disk, however, the infant repeatedly placed the bottle in his mouth, chewed on it, sucked at it briefly, only to withdraw it and repeat the sequence. It appeared that the training odor acted as a stimulus to initiate a feeding sequence, even though the child did not actually ingest any water. In adult animals, Weingarten has provided an important demonstration that auditory stimuli or signals paired with feeding can themselves come to induce ingestion, even in animals that are sated (Weingarten, 1983, 1984). These data suggest that an olfactory stimulus can serve as a signal to initiate a feeding sequence.

Irrespective of the mechanism, these data have positive implications for using this technique to aid parents and infants during changes in feeding regimens. For example, infants often reject the electrolyte solutions (i.e., Pedialyte, Ricealyte) offered when they are in danger of dehydration from gastro-intestinal illnesses. Manufacturers have attempted to counteract this by adding citrus, bubble gum, and grape scents to the solutions, but these odors may have little relevance to infants who have only experienced the odors of their own formulas while feeding. Using Flavor-Dots to add an odor to the bottles of formula-fed infants before they become ill might increase their subsequent acceptance of an electrolyte solution scented with a similar odor. Another application may be in facilitating the transition to another formula. For example, to adults, the protein hydrolysate formulas offered to infants who are intolerant of milk- or soy-based formulas are unpleasant tasting and smelling, and 7–8-month-old infants are reluctant to try the new formula (Mennella & Beauchamp, 1996b). Several days in advance, parents could introduce olfactory conditioning before making the transition to a new diet, and then pair the trained odor with the new diet to decrease its novelty and increase its intake. The olfac-

tory conditioning might entice the child to sample the new diet, after which positive postingestive changes might ease the final transition.

NOTES

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