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Sensitive period in flavor learning: effects of duration of exposure to formula flavors on food likes during infancy

Julie A. Mennella and Sara M. Castor

Monell Chemical Senses Center, Philadelphia, PA 19104-3308

Abstract

Background & Aims—Emerging research has revealed the existence of periods in which the developing brain has heightened sensitivity to environmental influences. We discovered a sensitive period, <4 months of age, when exposure to the flavor of extensively hydrolyzed protein hydrolysate formulas (ePHF) determines its hedonic tone. This formula has pronounced bitter, sour, and savory tastes compared to cow-milk-based formulas (CMF). This study aimed to determine the effects of duration of exposure during the sensitive period on a food containing an exemplar of the savory flavor.

Methods—Formula-fed infants were randomized into four groups at age 0.5 months: one control group fed the control cow-milk formula (CMF) for 8 months, and three groups fed ePHF for 1, 3, or 8 months and CMF otherwise. When infants were 8.5 months, their acceptance of a savory and plain broth was measured.

Results—Infants fed hydrolysate formula for 3 or 8 months, but not 1 month, showed greater acceptance of the savory broth relative to the plain broth ($p < 0.01$) and consumed it at a faster rate ($p = 0.01$).

Conclusions—The duration of flavor exposure affects infants' earliest responses to foods: a 3-month exposure to this formula shifted the hedonic tone for savory flavor.

Keywords

taste; formula; preferences; infant feeding; glutamate; hydrolyzed casein

1. Introduction

Rapid rates of growth during the first year affect later health outcomes, leading many to argue that early life should be the focus for both preventive intervention and scientific inquiry¹. Although nutrition during this early sensitive period may affect health outcomes throughout the lifetime², relatively less attention has been paid to another important feature

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Address for correspondence: Julie A. Mennella, PhD, 3500 Market Street, Philadelphia, PA 19104-3308, phone: 267-519-4880, fax: 215-898-2084, mennella@monell.org.

CONFLICT OF INTEREST

There is no conflict of interest from authors related to this study.

STATEMENT OF AUTHORSHIP

J.A.M. was involved in study design and implementation, data interpretation, and manuscript writing. S.M.C. assisted in data collection and analyses and manuscript writing. All authors read and approved the final manuscript.

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of nutrition: how humans learn to like the flavors of foods. Although relationships exist between food habits in childhood and later in life³, these data do not address how and when experiences with food flavors affect later preferences. In particular, they do not shed light on whether the maturation of the senses that underlie flavor, as for other senses,⁴ depends upon proper stimulation during well-defined periods of development, commonly referred to as sensitive periods.

The absence of a robust experimental paradigm, like that employed for other sensory systems (e.g., vision⁵, audition/language⁶) and other animals^{7,8} has inhibited progress in understanding whether human flavor programming exhibits age-related changes in functional plasticity, commonly referred to as sensitive periods. To address this gap, we have investigated a model system that exploits the naturally occurring flavor variation in infant formulas to characterize the sensitive period in flavor learning^{9–11}. Using infants' responses to the distinct flavor of extensively hydrolyzed protein hydrolysate formula (ePHF), we identified a sensitive period, <4 months of age, when exposure (or lack thereof) to the flavor determines its hedonic tone. Infants fed this formula during the first few neonatal months preferred its flavor compared to infants without such exposure⁹. One and 3 months of exposure led to a similar level of acceptance of ePHF at 7 months. These infants were less accepting of the formula flavor than infants with 7-months of exposure, suggesting a dosing effect.

The distinctive flavor of ePHF is partly due to abundant free amino acids, most notably glutamate¹², which occurs naturally in many foods, such as broths. While we have some evidence from non-randomized studies that at weaning, infants whose parents chose to feed them ePHF (usually because of colic) are more accepting of savory, bitter, and sour tastes than were infants whose parents chose to feed them cow's milk formula (CMF)¹³, we do not know if the altered preferences are due to experiences with the formula *per se*. In this study, we conducted a randomized control trial to investigate how the duration of exposure to the formula flavor of ePHF impacted the infants' acceptance of a food containing an exemplar of a savory flavor during weaning. To this end, we compared acceptance of broth with and without glutamate in experimental groups of infants randomly assigned to be fed ePHF for 1, 3, or 8 months or never exposed to its flavor, to determine the dosing effects of exposure during the sensitive period.

2. Methods

Subject Groups

Forty-seven mothers who were formula feeding their infants were recruited during 2006–2009 (41.3% Black, 21.7% White, 19.6% Hispanic, 17.4% mixed race/other) to participate in a randomized experiment to study sensitive periods in learning⁹. When infants were 2 weeks, they were randomized into one of four groups (Fig. 1): infants fed cow-milk-based formula (CMF; Enfamil; Mead Johnson Nutritionals, Evansville, IN; CMF Control, n=13) for 8 months or fed ePHF (Nutramigen; Mead Johnson Nutritionals; Evansville, IN) for 1 (1M ePHF, n=11), 3 (3M ePHF, n=11), or 8 (8M ePHF, n=11) months and CMF otherwise. Ten additional dyads were excluded because the mothers did not complete the study (n=9) or the infant's formula was prepared with broth (n=1).

Mothers were unaware of the hypothesis, group assignment, and formula assigned each month. The Office of Regulatory Affairs at the University of Pennsylvania approved all procedures used in this study, and informed consent was obtained from each woman before study entry. Mothers were compensated for their participation. This trial was registered at clinicaltrials.gov as NCT00994747.

Testing Procedures

The broth was prepared by mixing 1.88g vegetable consommé powdered broth of which glutamic acid has been removed (Ajinomoto Inc., Kanagawa, Japan) with 500 mL room-temperature distilled water (plain broth) or a similar volume of a 0.4% (0.021 M) monosodium glutamate (L-glutamic acid monosodium salt monohydrate, Sigma-Aldrich, St. Louis, MO) solution (savory broth).

When infants were 8.5 months, the dyad was tested at Monell on 2 separate days. The test method controlled for several factors to allow evaluation of infant hedonic response independent of the caregiver and experimenter¹⁴. Testing was conducted under naturalistic conditions in which infants determined the pacing and duration of feeding and occurred at the same time of day for each infant, approximately an hour before the infant's next scheduled feed. Feeding ended when the child rejected the food at least three consecutive times, exhibiting such rejection behaviors as turning his or her head away, pushing the bottle away, or crying, or becoming playful. During test sessions, infants were videotaped while mothers oversaw the child's self-paced feeding. Mothers refrained from talking to minimize influence on infant behaviors, and the experimenter sat behind the video camera, out of view of the dyad.

The broth was presented in 2-ounce bottles (Similac Volu-Feed Nurser, Abbott Laboratories, Columbus, OH) that were an appropriate size for self-feeding (Fig. 1). In counterbalanced and randomized order, the child fed the plain broth on one day and savory broth on the other. The amount consumed was determined by weighing each bottle immediately before and after each feed. Immediately after each session, mothers rated their infant's enjoyment of the broth on a 9-point scale (9=extreme like). From the videotapes, we determined the length of the feed, from which we calculated rate of feeding (g/min). Mothers were queried about their infant's feeding history, and infants were measured and weighed, from which weight-for-height z-scores were calculated using World Health Organization Anthro software (version 2.02).

Statistical Analyses

Dependent measures included total intake (g) and rate (g/min) of each feed and mothers' rating of their infant's enjoyment of the broth during each test session. For each infant and each measure, we calculated a relative score by dividing infant response to savory broth by responses to savory broth plus plain broth, thus eliminating absolute differences in responses that may be due to individual differences.

Separate analyses of variance (ANOVA) were conducted with experimental group as the between-subjects factor and with *a priori* contrasts specified to focus on differences between experimental groups and the control group. Weight-for-height z-scores were covaried for all analyses to account for effects of body weight. Analyses were conducted with STATISTICA (version 8; StatSoft, Tulsa, OK), and $p < 0.05$ indicated statistical significance. For multiple comparisons, p-values were Bonferroni corrected (significance at $p < 0.017$). All summary statistics are expressed as mean $\pm$ SEM.

3. Results

Groups did not differ in age of first exposure to table foods ($p=0.50$; Table 1). At the time of testing, all were eating table foods, but only 34% ($n=14$) had ever experienced soup; these infants fed soup only 3.1 ± 0.7 times before testing. We found significant effects of experimental group on relative intake ($p=0.007$) and rate of feeding ($p=0.01$). Infants randomized to feed ePHF for 3 or 8 months ate more of the savory broth ($p=0.002$ and 0.005 , respectively), and consumed it at a faster rate relative to the plain broth ($p=0.004$ and

0.03, respectively), than the CMF control group (Fig. 2). The 1M ePHF experimental group and the control group did not differ, and maternal ratings of infant enjoyment of the savory relative to plain broth showed no significant group effects, suggesting mothers were unaware of the differences in acceptance.

4. Discussion

How many months infants fed ePHF influenced their acceptance of a savory-flavored broth at weaning. Because hydrolysate formulas have higher levels of glutamate and taste more savory than do milk-based formula, infants feeding ePHF have more exposure to these tastes and are thus provided with opportunities to learn to prefer them. Our data suggest a possible dosing effect: duration of exposure to ePHF influenced acceptance of glutamate-containing broth. The amount of exposure to the flavor of this formula required to shift the hedonic tone for glutamate in a food appears to be about 3 months. The effects of early exposure appear to be particularly persistent, leading to heightened preferences several years after children's last exposure to the formula for foods containing similar flavor notes (e.g., sulfur volatiles) such as broccoli¹⁵.

This model system of research investigation sheds light on sources of individual differences in taste. Although only the savory exemplar was examined in this study, the general principles likely have much broader significance. The adaptive reason for this age-related plasticity may reflect the importance of infants becoming familiar with and accepting of flavors that their mothers consume, which are transmitted through breast milk.¹¹ Because not all formulas have the same flavor, different formulas will have different effects on early flavor programming^{9,10}. Our findings suggest that flavor experiences in both breast milk¹¹ and formula (present results) later predispose children to be more accepting of foods containing these flavors.

Contrary to some recommendations that infants should be weaned early to develop a liking for foods (see¹⁶ for discussion), infants do not need solid food—they can learn from mother's milk. A wide variety of flavors are available through breast milk and better reflect the flavor palate the child will enter than do weaning foods or infant formulas, creating a better foundation for varied flavor attachment beyond weaning. These results implicate breastfeeding, and a varied diet for the nursing mother, in acceptance of varied foods at weaning and beyond and suggest that breastfeeding mothers may have to eat a food for at least 3 months in order for their nursing infants to experience and learn to like the taste of that food. This hypothesis is currently under investigation.

These results, from the first randomized clinical trial establishing a sensitive period for flavor preference during infancy, indicate that early flavor experiences alter children's food preferences. In light of these results, recommendations for (a) a varied healthy diet for pregnant and breastfeeding mothers and (b) repeated introduction of healthy foods of varied flavors at weaning may lead not only to healthier mothers and infants but also to increased hedonic appeal of healthier foods later in life. The data also raise an important distinction between the two primary modes of infant feeding. Unlike breastfed infants, formula-fed infants attach to flavors that may differ from their mother's flavor preferences and hence differ from what will most likely be fed to them during childhood. More knowledge of the type of flavor learning that is occurring during formula feeding and communication of this knowledge to the caregiver is needed.

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Non-standard abbreviations

ePHF	extensively hydrolyzed protein hydrolysate formula
CMF	cow milk formula

References

- Lucas A. The developmental origins of adult health and well-being. *Adv Exp Med Biol.* 2005; 569:13–15. [PubMed: 16137100]
- Hanson MA, Gluckman PD. Developmental origins of health and disease: new insights. *Basic Clin Pharmacol Toxicol.* Feb; 2008 102(2):90–93. [PubMed: 18226060]
- Skinner JD, Carruth BR, Wendy B, Ziegler PJ. Children's food preferences: a longitudinal analysis. *Journal of the American Dietetic Association.* 2002; 102:1638–1646. [PubMed: 12449287]
- Epelbaum M, Milleret C, Buisseret P, Dufier JL. The sensitive period for strabismic amblyopia in humans. *Ophthalmology.* Mar; 1993 100(3):323–327. [PubMed: 8460000]
- Lewis TL, Maurer D. Multiple sensitive periods in human visual development: evidence from visually deprived children. *Dev Psychobiol.* 2005; 46(3):163–183. [PubMed: 15772974]
- Ruben RJ. A time frame of critical sensitive periods in language development. *Acta Otolaryngology.* 1997; 117:202–205.
- Sullivan RM, Holman PJ. Transitions in sensitive period attachment learning in infancy: the role of corticosterone. *Neurosci Biobehav Rev.* 2010; 34(6):835–844. [PubMed: 19931556]
- Woo CC, Leon M. Sensitive period for neural and behavioral response development to learned odors. *Developmental Brain Research.* 1987; 36:309–313.
- Mennella JA, Lukasewycz LD, Castor SM, Beauchamp GK. The timing and duration of a sensitive period in human flavor learning: a randomized trial. *Am J Clin Nutr.* May; 2011 93(5):1019–1024. [PubMed: 21310829]
- Mennella JA, Griffin CE, Beauchamp GK. Flavor programming during infancy. *Pediatrics.* 2004; 113(4):840–845. [PubMed: 15060236]
- Sausenthaler S, Koletzko S, Koletzko B, et al. Effect of hydrolysed formula feeding on taste preferences at 10 years. Data from the German Infant Nutritional Intervention Program Plus Study. *Clin Nutr.* Jun; 2010 29(3):304–306. [PubMed: 20110140]
- Ventura AK, San Gabriel A, Hirota M, Mennella JA. Determination of free amino acid content in infant formulas. *Nutrition and Food Science.* In Press.
- Mennella JA, Forestell CA, Morgan LK, Beauchamp GK. Early milk feeding influences taste acceptance and liking during infancy. *Am J Clin Nutr.* 2009; 90(3):780–788S.
- Forestell CA, Mennella JA. Early determinants of fruit and vegetable acceptance. *Pediatrics.* 2007; 120(6):1247–1254. [PubMed: 18055673]
- Mennella JA, Kennedy JM, Beauchamp GK. Vegetable acceptance by infants: effects of formula flavors. *Early Hum Dev.* 2006; 82(7):463–468. [PubMed: 16469455]
- Fewtrell M, Wilson DC, Booth I, Lucas A. Six months of exclusive breast feeding: how good is the evidence? *British Medical Journal.* 2011; 342(c5955):209–212.

Figure 1. Experimental groups

The groups refer to the duration (months) fed protein hydrolysate formula (ePHF; dark grey boxes) or cow milk formula (CMF; light grey boxes). The months refer to the age of the infants at the beginning of each monthly cycle and then the age of infants (8.5 months) when test meals were conducted (crosshatched bars). On 2 separate days, infants were videotaped as they fed a plain or savory broth during infant-led feeding conditions. The broth was presented in 2-ounce bottles that were an appropriate size for self feeding. The mother of the subject provided consent for use of the photograph.

Figure 2. Intake

Amount of savory broth relative to plain broth consumed by 8.5-month-old infants during infant-led feeding. Repeated measures ANOVA with *a priori* planned comparisons between experimental groups and CMF control indicated that infants with 3 and 8 months of exposure to ePHF consumed significantly more of savory broth relative to plain broth than control group. *Significantly different from the CMF control group, $p < 0.017$.

Table 1

Demographics^a

Characteristics	Experimental Group Name				p-value ^c
	CMF Control ^b (n=13)	1M ePHF (n=11)	3M ePHF (n=11)	8M ePHF (n=11)	
Infants					
Sex (% female)	46.2% (6)	45.5% (5)	45.5% (5)	36.4% (4)	0.99
Age at testing (mos)	8.59 ± 0.09	8.55 ± 0.10	8.50 ± 0.10	8.52 ± 0.10	0.91
Ethnicity (%)					0.98
African American	38.5% (5)	36.4% (4)	45.5% (5)	45.5% (5)	
Caucasian	30.8% (4)	9.1% (1)	18.2% (2)	27.3% (3)	
Hispanic	23.1% (3)	18.2% (2)	27.3% (3)	9.1% (1)	
Other/more than one	7.7% (1)	36.4% (4)	9.1% (1)	18.2% (2)	
Weight for height z-score	0.79 ± 0.25	0.76 ± 0.28	0.09 ± 0.28	0.29 ± 0.28	0.19
Age of introduction to solid foods (mos)	4.39 ± 0.47	3.58 ± 0.51	4.58 ± 0.51	4.42 ± 0.51	0.52
Prior exposure to soup (%)	30.8% (4)	27.3% (3)	54.5% (6)	9.1% (1)	0.32
Mothers					
Age (yrs)	27.3 ± 2.2	26.2 ± 2.4	27.5 ± 2.3	27.8 ± 2.3	0.97
Parity (% multiparous) ^d	58.3% (7)	40.0% (4)	45.5% (5)	18.2% (2)	0.81
Education (% college) ^d	25.0% (3)	40.0% (4)	27.3% (3)	36.4% (4)	0.99

^aData are presented as means ± SEM or percent (n).^bCMF Control group was fed ePHF for 0 months.^cYates Chi Square or ANOVA, when appropriate.^dData missing for 1 dyad in CMF Control and 1 dyad in 1M ePHF group.