

Short communication

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

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SUMMARY

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 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 ePHF shifted the hedonic tone for savory flavor.

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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 inquiry.¹ Although nutrition during this early sensitive period may affect health outcomes throughout the lifetime,² relatively less attention has been paid to another important feature of nutrition: how humans learn to like the flavors of foods. Although relationships exist between food habits in childhood and later in life,³ 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., vision,⁵ 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 exposure.⁹ 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 glutamate,¹² 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

Abbreviations: ePHF, Extensively hydrolyzed protein hydrolysate formula; CMF, Cow milk formula.

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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

2.1. Subject groups

Forty-six 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 learning.⁹ 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 (1 M ePHF, $n = 11$), 3 (3 M ePHF, $n = 11$), or 8 (8 M 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.

2.2. Testing procedures

The broth was prepared by mixing 1.88 g 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 experimenter.¹⁴ 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

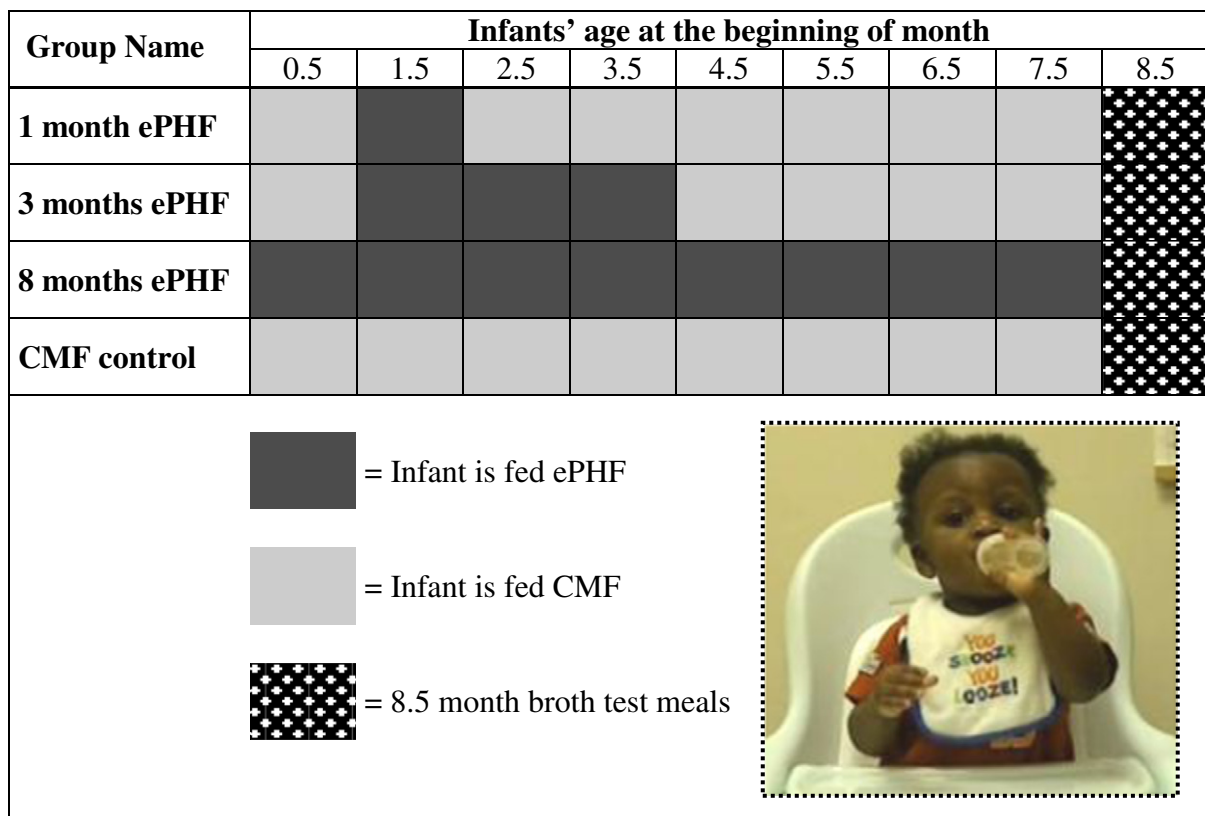


Fig. 1. Experimental groups. The groups refer to the duration (months) fed protein hydrolysate formula (ePHF; dark grey boxes) or cow milk formula (CMF; light grey boxes). The months refer to the age of the infants at the beginning of each monthly cycle and then the age of infants (8.5 months) when test 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.

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