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No evidence of flavour-nutrient learning in a two-week 'home exposure' study in humans

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Abstract

Flavour-nutrient learning is robust in animals but remains elusive in humans. Recent evidence suggests flavour-nutrient learning may be more likely to occur with beverages that contain relatively few calories (compared to no calories), while others show that learned associations can influence satiation, without an effect on preference. The objective of this research was to determine whether acquired liking for a caloric drink could be observed in a 'home learning' context over 2 weeks, and whether it is impacted by viscosity. In combination, we also explored changes in learning relating to fullness and expected satiety. In a double-blind study, participants ($N = 83$; BMI = 23.3 kg/m^2) were randomly allocated to one of four groups differing in either calories (0 kcal vs. 112.5 kcal) or viscosity (low vs. high) and consumed a novel-flavoured drink over 15 days. Measures of flavour (10 ml sample) and beverage liking, grip force (a measure of beverage reward value), fullness, and expected satiety were taken at the start and the end of the study. While the high-viscous beverages were less liked ($M = 40.3$ mm, $SD = 24.7$) than the low viscous beverages ($M = 64.4$ mm, $SD = 15.3$; $p = .022$), there was no evidence that repeated exposure to a calorie-containing beverage impacted subsequent liking for the flavour ($p = .115$) or for the beverage ($p = .448$), grip force (ps $> .26$), fullness, and expected satiety (ps $> .12$). Accordingly, we conclude that we found no evidence of flavour-nutrient learning and flavour-satiety learning. This null finding accords with previous observations indicating that humans do not acquire flavour-nutrient associations as readily as some non-human animals.

Introduction

Understanding the underlying processes that support dietary learning can help us to appreciate aberrant decision making, especially decisions associated with obesity. One such process involves the formation of an association between the post-ingestive effects of a food (the unconditioned stimulus - US) and its sensory characteristics (the conditioned stimulus - CS). After repeated CS-US pairings, the CS becomes preferred or liked more than other unpaired CSs. Sometimes, this is referred to as 'flavour-nutrient-hedonic learning' (FNL-H; (Yeomans, 2012).

There is an abundance of research showing that non-human omnivores can learn these associations rapidly (Myers, 2018; Sclafani & Ackroff, 2004). However, evidence in humans is equivocal. In a review by Yeomans (2012), it was noted that FNL-H was detected in only 9 of 14 human studies, and to varying degrees. One explanation for the discrepancy between animal and human findings relates to the novelty of foods. In studies using laboratory-reared animals, the relatively homogenous and well-controlled food history (e.g., lab chow) ensures that CSs are novel (Pérez, Fanizza, & Sclafani, 1999), which limits latent inhibition (i.e., the inhibition of learning caused by prior exposure to a CS). By contrast, in humans, latent inhibition might be more likely because true CS novelty is difficult to achieve, and as noted by Yeomans (2012), only 25% of studies explicitly assessed novelty. Furthermore, modern Western diets are likely to comprise numerous foods and food varieties, which may compromise opportunities to observe learning in controlled studies (Attuquayefio et al., 2016; Hardman, Ferriday, Kyle, Rogers, & Brunstrom, 2015; Martin, 2016). Notwithstanding this point, we also note that FNL-H was also absent in a study of semi-nomadic pastoralists who had not been exposed to a varied Western diet (Brunstrom, Rogers, Myers, & Holtzman, 2015), adding to a body of research questioning the reliability of human FNL-H under controlled conditions.

Another important factor to consider is the energy content of the test stimuli. Very low-calorie foods may be insufficient to trigger the post-ingestive signalling that is needed for flavour-nutrient associations to form, while calorie-rich foods may be especially satiating and/or aversive (Yeomans, 2012). For this reason, some have suggested a potential 'sweet spot' – an optimal energy content that maximises the likelihood of observing FNL-H (Veldhuizen et al., 2017; Yeomans, 2012). Consistent with these concerns about novelty and energy content, FNL-H was recently demonstrated using self-report liking ratings, but only when truly novel flavours were paired with a low-calorie solution [~112.5 kcal] (Veldhuizen et al., 2017). The authors hypothesised that learning (i.e., increased liking for the flavour paired with 112.5 kcals) was evident at this caloric level due to an optimal matching between sweetness and calories, suggesting that dietary learning may be maximised when such conditions are met.

Another form of dietary learning occurs when a novel flavour (CS) becomes associated with satiation (US), such that the CS develops the capacity to modify meal size (learned satiation) or post-meal satiety (learned satiety). Learned satiation and satiety likely represent the same underlying associations, but with different behavioural outcomes, and they are both regarded as examples of flavour-nutrient satiety learning (FNL-S) (Yeomans, 2012). Interestingly, while satiation and satiety are generally not reinforcing in non-human animals (Sclafani & Ackroff, 2004), and do not promote human preference learning (Brunstrom, 2007), foods may be selected over others based on their ability to satiate (expected satiation) and to delay hunger (expected satiety) (Brunstrom & Rogers, 2009; Wilkinson & Brunstrom, 2009). Indeed, it has been suggested that the behavioural outcomes of FNL-S may manifest in the opposite direction to FNL-H (Yeomans, 2012), with the former decreasing intake for the satiating food to avoid over-satiation, while the latter becomes liked thereby increasing intake for the caloric food. In humans, increasing the viscosity of a food facilitates learned satiation

(Mars, Hogenkamp, Gosses, Stafleu, & De Graaf, 2009), slows eating rate, and increases satiation and satiety (McCrickerd, Lim, Leong, Chia, & Forde, 2017; Zhu, Hsu, & Hollis, 2013). Meanwhile, FNL-H is typically indicated by increased reported liking or intake for the caloric food or beverage. One might therefore expect that learning about the post-ingestive consequences of caloric viscous foods will lead to increased liking, but decreased consumption. Thus, the viscosity of the CS may be important for observing different types of dietary learning. Potentially, animals use viscosity as a predictor of the energy content of food (Davidson & Swithers, 2004) and viscous foods are generally perceived as more desirable (Drewnowski, 1992). Therefore, we tested how viscosity might influence acquisition of FNL-H. One possibility is that more viscous foods promote FNL-S, while less viscous foods promote FNL-H. If this is the case, viscosity may moderate the extent to which FNL-H is observed. Indeed, one might expect the post-ingestive effects of more high viscosity foods to be rewarding and hence interact additively with calories, with the greatest shifts in FNL-S and FNL-H evident when both calories and viscosity are combined. To test these hypotheses, participants were randomised into one of four groups differing in either calories [0kcal or 112kcal] or viscosity [low or high] and underwent a double-blind 'home exposure' study conducted over two-weeks. To promote exposure to novel flavour-beverage pairings, we selected flavours that were regarded as novel and, drawing on parameters from previous research (Mars et al., 2009; Veldhuizen et al., 2017), manipulated the energy content and the viscosity of a liquid drink. We tested whether changes in FNL-H (liking, food reward) or FNL-S (appetite, expected satiety and satiation) were related to energy or viscosity.

Method

Design

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The study employed a 2 x 2 x 2 design, with two between factors (groups) and one within factor (time). Participants were randomly allocated to one of four conditions based on caloric load and viscosity – no calories/low viscosity (NC-LV); no calories/high viscosity (NC-HV); calories/low viscosity (C-LV); calories/high viscosity (C-HV). Participants in each condition were required to consume a 340mL beverage, once daily over 15 consecutive days. The study had 3 phases: pre-study testing, at-home beverage consumption, and post-study testing. Materials and procedures are detailed below.

Participants

Participants (*N*= 107) were recruited from the population of staff and students at the University of Bristol, and from the local community. Volunteers were eligible to participate if they: 1) were aged between 18 and 70 years, 2) were fluent in English, and 3), were prepared to consume food-grade additives that are found in commercially available foods (*e.g.*, thickening agents, protein/carbohydrate powders, non-nutritive sweeteners, flavourings, and colourings). The research was approved by the University of Bristol Faculty of Science Human Research Ethics Committee (ref: 23111759761). A power analysis revealed that a total of 76 participants (n = 19 per group) would be required to detect a medium effect size (f=0.2) at 80% power for a planned 2-way ANOVA repeated-measures analysis.

Materials

Beverages and flavours

All 340-ml beverages and flavour tests contained a non-nutritive sweetener, sucralose (0.0078% w/v), citric acid (0.1% w/v) food colouring (red; McQueen, Sainsbury's UK) and filtered water. The eight flavours were identical to those used by Veldhuizen et al. (2017) and included 0.1% horchata, 0.1% lulo, 0.2% yuzu. 0.1% papaya, 0.1% chamomile, 0.1% aloe

vera, 0.1% mamey, and 0.2% maqui berry (Bell Labs Flavors and Fragrances, IL, USA,

product numbers: 33.81940, 15.80182, 132.81478, 141.14606. 101.29478, 102.82506,

141.31243, 141.31480, 46.29969 and 13.32059). All beverages and flavour tests were the

same colour (red), regardless of flavouring. The flavour tests comprised 0.0078% g sucralose,

0.1% citric acid, food colouring, and the appropriate concentration of flavouring. Following

Veldhuizen et al. (2017), caloric beverages we formulated by adding 112.5 kcal of

maltodextrin (DE 19, Roquette Le Strem, France). The viscosity of the beverages was

manipulated by adding a commercially available food-grade thickening agent (0.36% w/v

tara gum). Beverages were prepared by one of the authors (TA) who was not in contact with

the participants over the two-week period, and all other researchers were blinded to the

beverage condition. Thus, the study was conducted double-blind.

Ratings

Participants rated overall intensity, sweetness intensity, liking, and thickness on a

100-mm visual analogue scale (VAS) anchored 'Not at all' to 'Extremely'. Liking ratings

also included a 'Neutral' anchor at 50 mm. Ratings of 'wanting more' (hereafter referred to

as wanting) were also taken (on a 100-mm VAS anchored by 'Not at all' and 'A lot')

alongside the more common 'liking' rating, as there is evidence that the former is different

from the latter, and is more sensitive than traditional 'wanting' ratings (Attuquayefio et al.,

2016; Stevenson, Francis, Attuquayefio, & Ockert, 2017). These ratings were used to provide

3 hedonic tests for the flavour, sample and beverage. The flavour test was an extinction test

designed to measure change in ratings for the flavour paired with nutrients and/or viscosity

(CS^+), while sample ratings were taken after a 10mL sample, and beverage ratings after

consumption of the beverage. Measures of appetite (hunger, fullness, and thirst) and satiation

(expected and reported satiation) were also taken on a 100-mm VAS anchored 'Not at all' to

'Extremely'.

Anthropometric and dietary measures

Measures of body mass index (BMI, kg/m^2), body-fat percentage and fat-free mass index (FFMI, kg/m^2) were derived using a measure of electrical impedance (Tanita Corporation: Body Composition Analyser, BC-418 MA III). We also used the Dutch Eating Behaviour Questionnaire (DEBQ) (Van Strien, Frijters, Bergers, & Defares, 1986) to measure three different types of trait eating style: restrained, emotional, and external eating.

Expected satiety task

We used an established protocol for a computer task measuring expected satiety using 11 food and beverage pictures (Brunstrom et al., 2016). Along with the test beverage, ten commonly consumed foods and beverages were used as stimuli (diet coke, sparkling water, orange juice, semi-skimmed milk, apricots, chocolate M&Ms, boiled egg, salted peanuts, boiled potatoes, and salt & vinegar crisps). In each of the 11 trials, one food was displayed on the left-hand side of the computer screen and a comparison food (pilau rice) was presented on the right. For each of the 11 comparison pairs, the participants were asked to "change the size of the portion on the right so that both foods will prevent hunger for the same amount of time." Changes to portion size were made in 20-kcal allotments, though this fact was not known to participants.

Grip force task

We measured grip force using a metal frame connected to a load cell that was interfaced with a computer. This hand-held device measured the force applied by hand grip while viewing an image of a food or beverage. Participants were twice presented images of the same ten picture stimuli and test beverage as above, in a random order. Each image was presented for 3.0 seconds, with an inter-stimulus interval of 2.0 seconds (22 trials in total). They were asked "How much do you want this food or drink?" and told to respond by

gripping with both hands the handheld device accordingly (squeezing harder the more they wanted the food). For each trial, a measure of cumulative force was obtained by calculating the area under the curve (AUC) represented by force and time. A second measure was also obtained reflecting the maximum peak force applied in each trial (Max). Together, these measures assessed willingness to work (i.e., grip force) for each stimulus, including the test beverage. In this way, handgrip force is argued to be a measure of beverage reward value (Ziauddeen et al., 2014). The hand grip force task taps into the same construct as liking and wanting (Arumäe et al., 2019), but has not been applied previously in the context of dietary learning.

Procedure

Participants attended two test sessions separated by 14 days (day 1 and day 15, respectively). Each session took approximately 30 minutes. Testing commenced at least two hours after their last meal (between 10am and 4pm) and took place at the same time of day for both sessions. Participants were instructed not to eat or drink anything except water for at least two hours prior to each session, and to consume the same (or similar) meal at the same time of day for each test session. Compliance with instructions was verified verbally by the experimenter upon arrival.

Day 1

A 10-ml sample of each of the eight flavours (without calories or thickener) was prepared for participants to consume in a randomised order (hereafter flavour tests). Crackers and water were provided as palate cleansers between each flavour test. Each flavour was rated on novelty, liking, wanting, overall intensity, and sweetness intensity. Participants were also asked if the flavour was novel (Yes/No). Novelty and liking ratings were used to determine eligibility and flavour allocation. To qualify for inclusion, participants had to rate

9

at least one flavour as both novel (responded 'Yes') and at least 'Neutral' (≥50mm) on the liking VAS. If no flavours were rated as novel or liked ≥50mm, the participant was excluded. If more than one flavour met these criteria, the flavour rated closest to 50 on the liking VAS was allocated as the flavouring for the beverage. Eligible participants were then randomly allocated to one of the four test conditions.

After conducting the flavour test, eligible participants consumed a mouthful of their allocated flavoured beverage from their allocated condition (e.g., C-HV) and provided ratings (hereafter sample ratings) of overall intensity, sweetness intensity, liking, wanting, thickness, and how filling they expected the beverage to be based on the sample (*i.e.,* expected satiation). Sample ratings allowed for measurement of expectations (i.e., expected satiation) and liking prior to changes related to satiation associated with beverage consumption (i.e., alliesthesia) (Cabanac, 1971). This was followed by the grip force and expected satiety tasks using the 10 foods and the allocated flavoured beverage (11 stimuli in total). Participants rated appetite (pre-consumption) and were instructed to consume the 340-ml beverage and then to rate (hereafter beverage ratings) its overall intensity, sweetness intensity, liking, wanting, reported satiation (i.e., how filling the beverage actually is), and thickness. The bottle was then removed and weighed, and participants completed another set of appetite ratings (post-consumption).

Participants were then asked to take home 13 sealed 340-ml bottles of the test beverage, which was prepared and collected from the lab on a weekly basis. To standardise at-home consumption as much as possible, participants were asked to: 1) store the beverages in a refrigerator to preserve beverage flavour and integrity; 2) to shake the chilled beverages for 15-20 seconds before consumption; 3) to drink the beverages at approximately the same time every day; and 4) to consume them within 10 minutes in isolation (i.e., without food) within a 20 minute window prior to or after a meal. Compliance was encouraged with daily

reminders, and daily questionnaires were issued to assess how much of the beverage had been consumed (0-100%). Bottles were returned to the lab on a weekly basis to be weighed.

Day 15

Participants completed the lab tasks as on day 1 (*i.e*., flavour test ratings, sample ratings, grip force task, expected satiety task, beverage ratings, and pre- and post-consumption appetite ratings). In addition to assessments of FNL-H and FNL-S, participants were asked to estimate the number of calories in the beverage (0-300 kcals), completed the DEBQ, and anthropometric measures (BMI, FFMI, and fat percentage) were obtained. An outline of this procedure is provided in Figure 1.
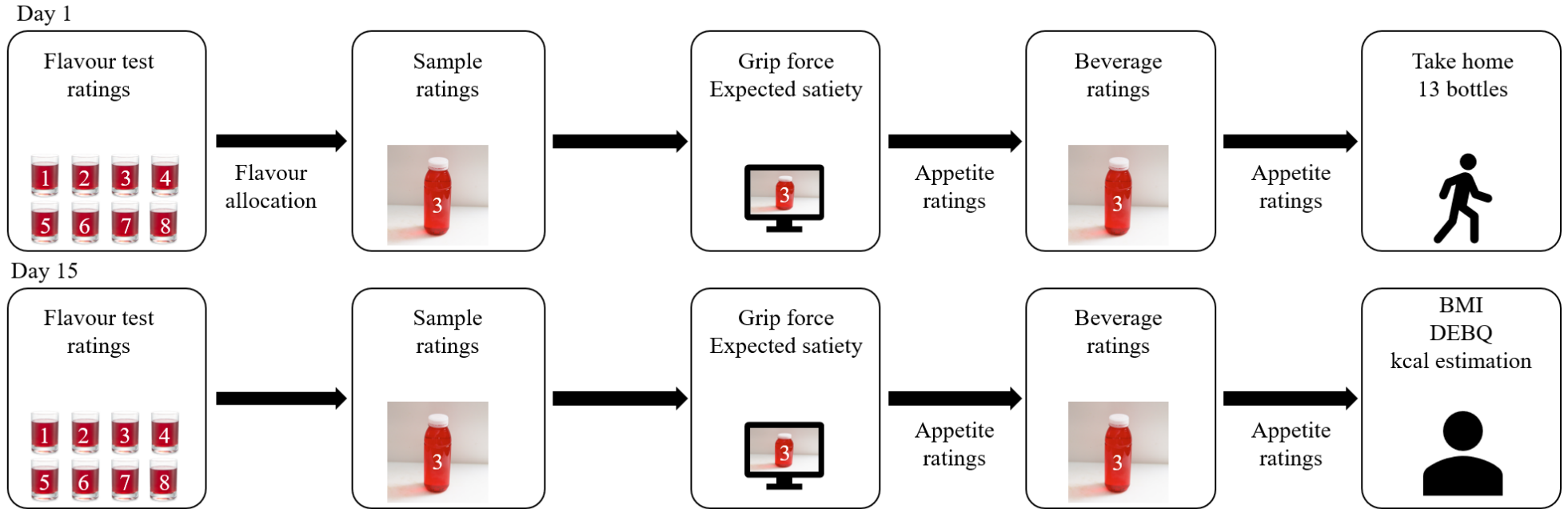
Figure 1. Outline of the procedure. All measures were taken on days 1 and 15. Flavour ratings on day 1 were used to determine participant

eligibility and to allocate a sample flavour to individual participants.

Analysis

Twenty-two participants did not meet the eligibility criteria and a total of 83 participants provided day 1 and day 15 data. Outliers were winsorised and missing values were imputed to the mean.

Evidence of FNL-H was assessed using three different ratings of liking (flavour, sample, and beverage) and two measures (AUC and Max) of grip force. As wanting and liking ratings were highly correlated (all $r > .6$), we only report findings relating to the liking ratings. A separate two-way repeated-measures ANOVA (Viscosity x Calories) was run on flavour, sample, and beverage liking ratings, with day (day 1 or day 15) as a within-subject factor. To determine if exposure to the allocated test beverage promoted an increase in liking for the paired flavour (CS$^+$), relative to other unpaired flavours (CS$^-$), we used a multi-level linear mixed-model with random intercept only to examine the interaction between paired status (CS$^+$/CS$^-$), viscosity (LV/HV), and calories (NC/C) on flavour liking ratings, with participant and flavour as levels. For the grip force task, we used the total area under the curve (AUC) and maximum force (Max) as measures of beverage reward value, which were entered into separate linear mixed models, with day, group, and test food (test beverage or not) as factors of interest, controlling for participant variability as a level.

Evidence of FNL-S was taken from ratings of hunger, fullness, satiation and expected satiation. These four ratings provided evidence of learned satiation – a behavioural outcome of FNL-S. Expected satiation ratings were taken from sample ratings, while reported satiation was taken from beverage ratings (following beverage consumption). In addition, for both days 1 and 15, we calculated the pre-to-post beverage consumption changes in hunger and fullness scores, and these change scores were subsequently analysed using repeated measures ANOVA (Viscosity x Calories). Change scores across time in the expected satiety task (i.e.,

1

29   learned satiety) were entered in a linear mixed model with day, group and test food entered as

30   levels, controlling for participant level.

31   **Results**

Participant characteristics

33   The final sample consisted of 83 young adults ($M = 25.6$ yrs, $SD = 8.4$), with females

34   comprising 54.2% of the total sample. Table 1 summarises the participant characteristics

35   across the four groups. Fat mass differed across groups (see Table 1), but there were no other

36   significant group differences (ps>.10). Intake compliance of the 340mL beverage was high

37   (>335mL) across the 15 days.

38

Table 1. Participant body composition and dietary behaviour
by condition

	Condition				
	NC-LV (n = 23)	NC-HV (n = 21)	C-LV (n = 20)	C-HV (n = 19)	p-value
Intake (mL)	336.8 (11.1)	340.0 (0)	336.9 (7.8)	338.6 (5.3)	.141
Age (yrs)	26.4 (8.6)	27.6 (10.4)	21.4 (2.4)	26.9 (9.2)	.268
Female %	56.5%	66.7%	40.0%	52.6%	.393
BMI (kg/m^2)	22.7 (8.6)	25.2 (5.4)	22.5 (3.4)	22.7 (2.9)	.137
Fat percent (%)	15.4 (4.6)[b]	19.7 (10.8)[a]	12.3 (4.9)[b]	15.2 (7.1)[a]	.764
FFMI (kg/m^2)	51.1 (9.3)	52.4 (11.6)	53.7 (11.5)	51.5 (10.5)	.392
DEBQ restraint	2.3 (0.7)	2.4 (0.8)	2.3 (0.9)	2.8 (1.0)	.590
DEBQ	2.5 (0.6)	2.5 (0.9)	2.6 (0.9)	2.5	.985

				emotional eating	(0.8)	
DEBQ external eating	3.5 (0.6)	3.4 (0.5)	3.5 (0.6)	3.4 (0.7)	.852	

39 a. Main effect of viscosity, $F(3,78) = 4.89$, $p = .030$; b. Main effect of calories, $F(3,78) =$
40 5.25, $p = .021$. NC-LV = no calorie/low viscosity; NC-HV = no calorie/high viscosity; C-LV
41 = calorie/low viscosity; C-HV = calorie/high viscosity.
42
43 *Calorie estimation*

44   The high viscosity beverages were rated as more caloric ($M = 173.9$ kcal, $SD = 59.9$)

45 than the low viscosity beverages ($M = 112.7$ kcal, $SD = 59.4$; $F(1,79) = 22.47$, $p < .001$),

46 partial $\eta^2 = .221$, and caloric beverages were also estimated to be more caloric ($M = 157.5$

47 kcal, $SD = 77.9$) than the non-calorie beverages ($M = 128.9$ kcal, $SD = 52.0$; $F(1,79) = 4.36$, p

48 $= .040$, partial $\eta^2 = .052$. The interaction between viscosity and calories was not significant (p

49 $= .139$). As this measure was only taken at day 15, it remains unclear whether changes in

50 calorie estimation occurred during the exposure period.

51 **FNL-H and FNL-S**

52   Across multiple measures, we found no evidence of FNL-H or FNL-S, and no

53 evidence that these forms of learning are moderated by viscosity. A summary of these

54 outcomes is presented in Table 2. For full statistical results, see Supplementary Materials.

55

Table 2. Summary of the significant increase (↑) or decrease (↓) and non-significant (×) results

	Day	Calories	Viscosity	Day x Cal	Day x Visc	Cal x Visc	Day x Cal x Visc
FNL-H							
Flavour liking	×	×	↓	×	×	×	×
Sample liking	↑	×	×	×	×	×	×
Beverage liking	↑	×	↓	×	×	×	×
Grip Force Max	↓	×	×	×	×	×	×
Grip Force AUC	↓	×	×	×	×	×	×
FNL-S							
Expected Satiety	×	×	↑	×	×	×	×
Expected Satiation	↑	×	↑	×	×	×	×
Reported Satiation	×	×	↑	×	×	×	×
Change in fullness	×	×	↑	×	↑	×	×
Change in hunger	×	×	↑	×	×	×	×
Calorie Estimation	×	↑	↑	×	×	×	×

57 Cal: Calories; Visc: Viscosity

FNL-H

Sample and beverage liking

Sample liking showed an increase across days, $F(1,79) = 6.33$, $p = .014$, partial $\eta^2 = .074$, but no significant main effects or interactions (ps > 0.16). Beverage liking scores also showed a similar overall increase between day 1 ($M = 46.8$ mm, $SD = 22.2$) and day 15 ($M = 52.8$ mm, $SD = 23.6$; $F(1,79) = 7.76$, $p = .007$, partial $\eta^2 = .089$). While HV beverages were liked less ($M = 37.6$ mm, $SD = 22.7$) than LV beverages ($M = 61.2$ mm, $SD = 16.4$; $F(1,79) = 37.07$, $p < .001$, partial $\eta^2 = .319$), no other main effects or interactions were found (ps > 0.24).
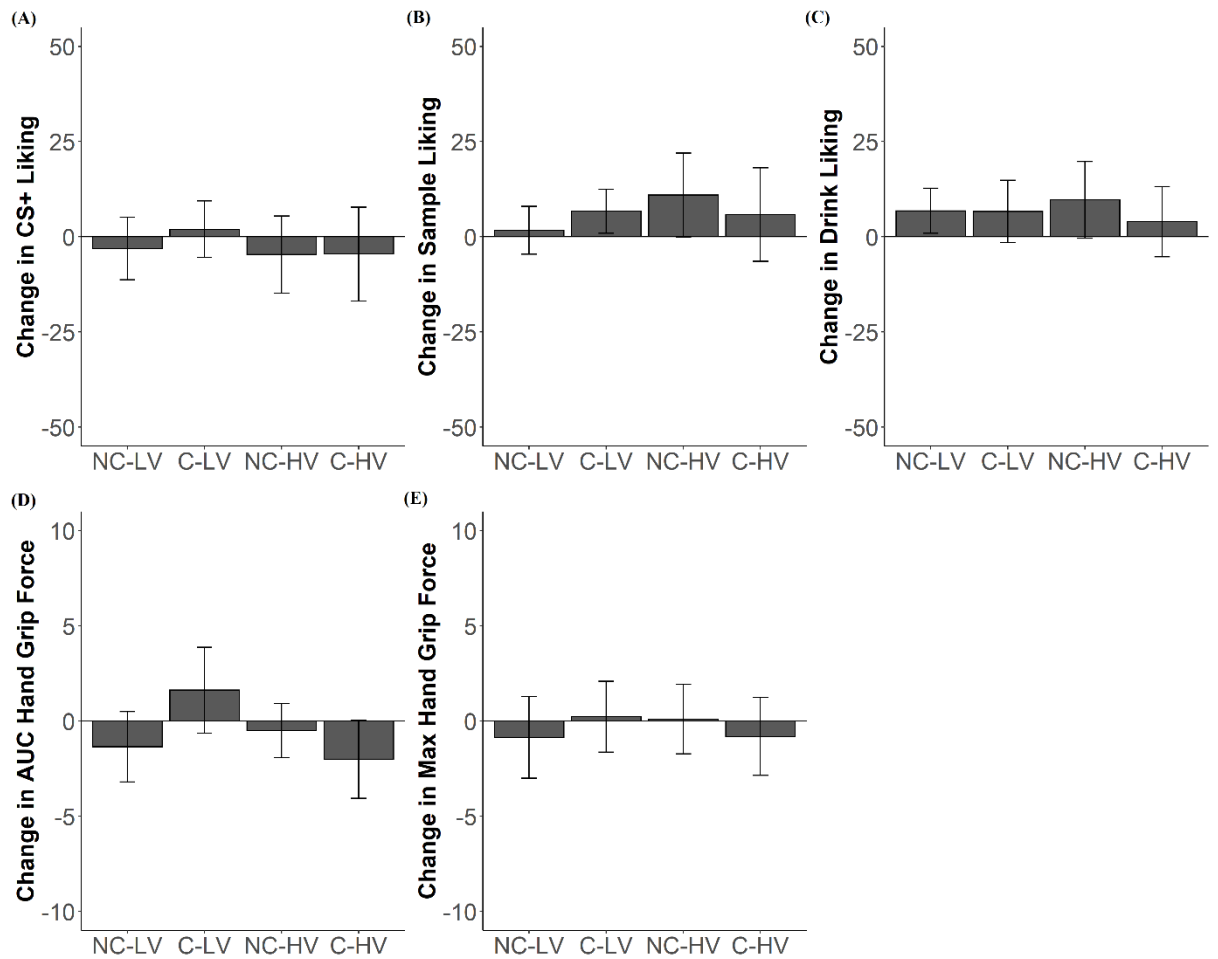
Flavour liking

Liking for the conditioned flavour (CS⁺) did not differ significantly across days and it was not impacted significantly by calories or viscosity (ps $> .27$; see Figure 2). In addition to

70   this, there were no group differences in liking for the conditioned flavour (CS$^+$) relative to the

71   other seven unconditioned flavours (CS$^-$; ps > .44).

72   *Grip force task*

73   For grip force, there was an overall reduction in handgrip applied to all foods across

74   days for AUC, $t(3406.73) = 5.31$, $p <.001$, and Max force, $t(3411.50) = 6.69$, $p <.001$. The

75   AUC for the test beverage, ($M = 74.5$ N, $SD = 92.2$) was significantly lower, $t(3384.93) =$

76   2.58, $p = .009$ than for all other foods, ($M = 85.3$ N, $SD = 107.9$). Similar results were found

77   for Max handgrip, $t(3385.3) = 2.69$, $p = .007$. However, there was no significant change

78   across days in AUC ($p = .25$) or Max force ($p = .66$) applied for test beverage. Importantly,

79   there was no interaction with day, viscosity, calories and test beverage, for AUC, $t(3332.9) =$

80   0.601, $p = .548$ and for Max, $t(3333.3) = 1.148$, $p = .251$.

81

Figure 2. Means (+/-2 SE) by group for measures of Flavour Nutrient Hedonic Learning (FNL-H) illustrated as change across time for: (A) Liking ratings for the test flavour (CS$^+$); (B) Liking ratings of the sample of the drink; (C) Liking ratings for the drink; (D) Area under the curve (AUC) of hand grip force applied for the test drink; and (E) Maximum hand grip force applied for the test drink.

FNL-S

Expected satiety task

There was a trend for greater expected satiety for the more viscous beverages ($M = 237.8$ kcals, $SD = 137.2$) relative to the less viscous beverages ($M = 135.4$ kcals, $SD = 117.7$;

92 $t(136.34) = 1.836$, $p = .068$). No other significant main effects or interactions were observed

93 (ps > .39 – See Figure 3).

94 *Expected and reported satiation*

95 The high viscosity beverages were expected to be more satiating ($M = 68.7$ mm, $SD =$

96 14.3) than the low viscosity beverages ($M = 45.2$ mm, $SD = 18.9$; $F(1,79) = 58.69$, $p < .001$,

97 partial $\eta^2 = .426$. Averaging across groups, expected satiation increased from day 1 ($M = 51.8$

98 mm, $SD = 24.6$) to day 15 ($M = 56.3$ mm, $SD = 23.2$; $F(1,79) = 4.31$, $p = .041$, partial $\eta^2 =$

99 .052), though this did not change across the exposure period or as a function of group (ps >

100 .13). Post-consumption, the HV beverages ($M = 73.4$ mm, $SD = 10.3$) were also rated as

101 more satiating than the LV beverages ($M = 55.7$ mm, $SD = 18.4$); $F(1,79) = 31.35$, $p < .001$,

102 partial $\eta^2 = .284$. No other main effects or interactions were found for reported satiation (ps >

103 .18). Interestingly, there was a significant negative correlation between day 15 ratings of

104 beverage liking and reported satiation ratings ($r(83) = -.235$, $p = .033$), suggesting that

105 satiating beverages were less liked.

106 *Change in hunger and fullness*

107 The high viscosity beverage led to a larger within-session change in fullness ratings

108 relative to the low viscosity beverage, $F(1,79) = 5.08$, $p = .027$, partial $\eta^2 = .060$, and the day

109 by viscosity interaction trended, $F(1,79) = 3.00$, $p = .087$, partial $\eta^2 = .037$, but no other main

110 effects or interactions were observed (ps > .37). Within-session changes in hunger also

111 showed a significant increase based on viscosity, $F(1,79) = 6.38$, $p = .014$, partial $\eta^2 = .075$,

112 but there were no other main effects or interactions (ps > .12).

7
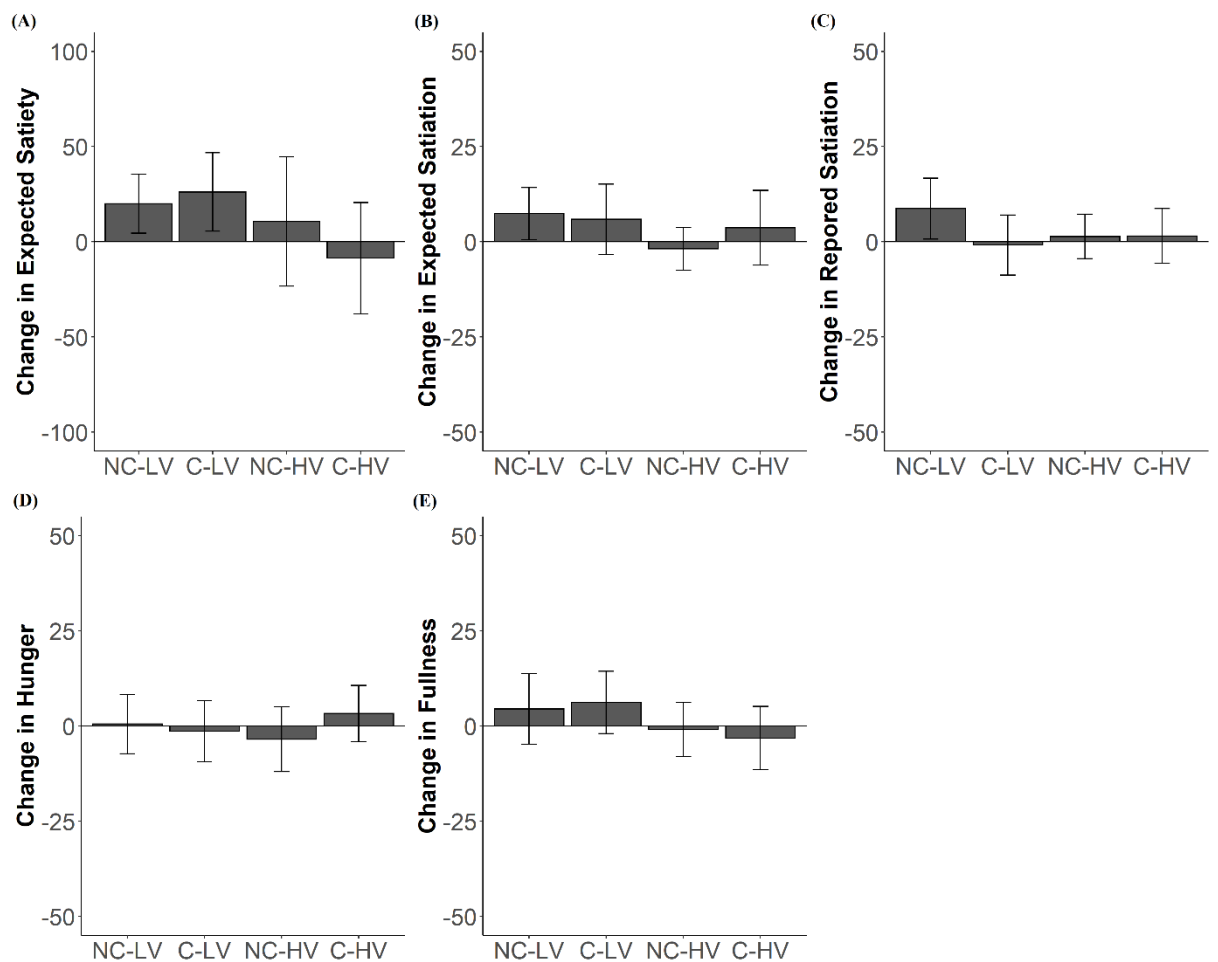
Figure 3. Means (+/-2 SE) by group for measures of Flavour Nutrient Satiety Learning (FNL-S) illustrated as change across time for: (A) Expected Satiety; (B) Expected Satiation ("*How filling you expect the test drink to be?*"); (C) Reported Satiation ("*How filling was the test drink?*"); (D) Pre-post drink consumption change in hunger; and (E) Pre-post drink consumption change in fullness.

Discussion

The objective of this experiment was to determine if FNL-H or FNL-S could be observed in an at-home exposure study using low-calorie test beverages, and whether this learning was moderated by viscosity. This was tested with novel flavours over an extended 2-week period using stimuli that have previously demonstrated FNL. Despite this, across

124   multiple measures, we found no evidence of either FNL-H or FNL-S, nor did we see an

125   interaction between calories and viscosity across measures believed to tap into FNL-H and

126   FNL-S, respectively.

127   From a Pavlovian perspective, the rewarding properties of the added nutrients or

128   viscosity should be imbued onto the novel CS (flavour) in the absence of the US (calories

129   and/or viscosity). Despite this, there was no evidence of changes in flavour liking as a

130   function of calories or viscosity. Over the exposure period, we observed an increase in liking

131   for all test (CS$^+$) flavours relative to untested (CS$^-$) flavours, which supports evidence for the

132   effect of 'mere exposure' that is often observed only in children (Holley, Farrow, & Haycraft,

133   2017). This is an interesting finding, as to the best of our knowledge, this has been difficult to

134   show in adults. Such exposure effects have been shown to increase fruit and vegetable intake

135   in children (Appleton, Hemingway, Rajska, & Hartwell, 2018), though the applications in

136   adults remain to be explored.

137   The handgrip task, which we believed would track changes in reward value of the

138   beverage over the exposure period, also showed no significant group differences based on

139   calories or viscosity. Interestingly, in agreement with (Arumäe et al., (2019), we found that

140   the behavioural measure of wanting (i.e., the hand grip task) was positively associated with

141   self-report measures of wanting (rs = .3-.5). We also observed group differences in liking for

142   the beverage – those with high viscosity were less liked than those with low viscosity, though

143   liking was not altered by exposure to calories. Differences in beverage liking across levels of

144   viscosity may have been driven by past learning, perhaps reflecting the relative novelty of

145   thickness in a beverage. Given that we observed group differences in beverage liking (albeit

146   in the opposite direction than expected) but not flavour liking, there is insufficient evidence

147   of flavour nutrient learning.

148   As expected, all measures of FNL-S (i.e., expected satiety, expected satiation,

149   reported satiation, change in fullness, change in hunger) were influenced by viscosity – a

150   finding consistent with previous research (Hogenkamp, Stafleu, Mars, Brunstrom, & de

151   Graaf, 2011; Mars et al., 2009). However, these measures did not interact with caloric load,

152   nor did they significantly change over the exposure period suggesting differences here are not

153   related to learning. Of note, the trend for greater changes in fullness for the high viscosity

154   beverage across days might suggest some evidence of FNL-S learning, though overall the

155   evidence is weak. In our current study, despite using a relatively long exposure period and

156   preselecting stimuli rated as novel, we did not find group-dependent changes in hedonic or

157   appetite measures that would reflect FNL-H or FNL-S. Similar null findings relating to

158   viscosity in FNL-H and FNL-S have been demonstrated by others (Gould, 2013).

159   The null findings observed here may be important for our understanding of the role of

160   FNL-H and FNL-S in human dietary learning. One explanation is that our measures lacked

161   sufficient sensitivity. Veldhuizen et al. (2017) were able to demonstrate FNL-H using very

162   similar methods and identical stimuli, but with a slightly different outcome measure – the

163   labelled hedonic scale (Lim et al., 2009). This leads to one of two conclusions. Either FNL is

164   robust but can only be expressed under very specific conditions and with very specific

165   outcome measures, or previous demonstrations reflect reporting bias. Either way, our findings

166   support mounting evidence that human FNL is unlikely to be observed in humans over

167   relatively short periods. Our findings add to the growing literature showing that flavour

168   nutrient conditioning in humans, especially adults, is quite challenging. To date, the

169   conditions under which FNL would be optimally demonstrated has not been elucidated.

170   It has been suggested that rapid FNL might be useful for other omnivores, but not to

171   the same extent for social humans who develop food preferences not just at the individual

172   level, but also through social interaction (Brunstrom & Cheon, 2018; Holley et al., 2017).

173   Indeed, there are several ways by which a food might become preferred, including social

174   transmission of affect via observational learning, FNL, flavour-flavour learning, and

175   cognitive top-down information. Consistent with this notion, observational learning has

176   robust effects on subsequent food preferences in pre-schoolers (Birch, McPhee, Steinberg, &

177   Sullivan, 1990) – an effect that appears to be moderated by the level of social connection

178   with the observer. Indeed, a recent systematic review found peer models were particularly

179   effective at increasing consumption of vegetables in 2-5 year olds, while flavour nutrient

180   conditioning had no significant effect (Holley et al., 2017). Similarly, a recent meta-analysis

181   of FNL studies found that increased exposure to novel vegetables increases liking and intake

182   in children, though effect sizes were small (Appleton et al., 2018). A further possibility is that

183   these processes interact. At an individual level the effects of FNL may be weak and may only

184   be observed under specific and tightly controlled conditions. Collectively, these learned

185   associations might have a powerful impact on preference, such that habits and behaviours are

186   shaped and transmitted culturally over long periods, using the collective 'wisdom' of the

187   group (Brunstrom & Cheon, 2018; Brunstrom et al., 2015). While recognising the speculative

188   nature of this proposition, it nevertheless fits with robust evidence of observational learning

189   and the many null or inconsistent findings in short-term FNL studies in humans (Brunstrom

190   & Cheon, 2018; Brunstrom et al., 2015; Holley et al., 2017; Yeomans, 2012). As such, FNL

191   may play a smaller role in the formation of human food preferences than is generally

192   supposed.

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238 z-

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240 4EBoJ6Vm7t-F-

241 hV968c08RCk5ZgrbqJgAcjNfFNPK6ux0G3nxtSWIA5fZcYCAiqc4oDSDtHZEjc6-

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